WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, July 07, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L1	random hexamer	2816
		DB=USPT,DWPI; PLUR=YES; OP=ADJ	
☐	L2	4683202.pn. and kit	1
☐	L3	(5837442.pn. or 5840487.pn.) and kit	2
		DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L4	hopkins-A$.in.	311
☐	L5	hopkins-A$.in.	311
☐	L6	L5 and random primer	1
☐	L7	L5 and random oligomer	0
☐	L8	((self anneal$)same primer)	151
☐	L9	L8 and (6-mer or 7-mer or 8-mer)	20
☐	L10	((prevent$ or inhibit$ or reduc$) same (self anneal$))	267
☐	L11	L10 and (random and (6-mer or 7-mer or 8-mer))	25
☐	L12	L10 and random hexamer	16
☐	L13	random hexamer and L8	18
☐	L14	L1 and (self-anneal$)	33
☐	L15	L1 and ((prevent$ or inhibit$ or reduc$)same (self-anneal$))	16
☐	L16	(hexamer or heptamer or octamer)same random	3047
☐	L17	L16 same (self-prim$ or self-anneal)	2
☐	L18	L16 same (dry or dried or freeze-dried or lyophili$)	152
☐	L19	(hexamer or heptamer or octamer)near (dry or dried or freeze-dried or lyophili$)	0
☐	L20	(hexamer or heptamer or octamer)same(dry or dried or freeze-dried or lyophili$)	432
☐	L21	l20 same random	152
☐	L22	L21 and primer extension	37
☐	L23	L21 and PCR	148

END OF SEARCH HISTORY

mixture kept at room temperature 16 hrs., the solvent is distilled and the residue 30
min. heated at 140°, then dissolved in III 100 parts, washed, and distilled yields I(R = 1-methylcyclohexyl, R' = Me3C) (IV), crystals from III, m. 89-91°, b0.1 200-25°. Other examples of I, prepared like IV (R and R' given): Me3CCH2CMe2, 1-methylcyclohexyl, b1.0 235-45°; and Me3C, 1-methylcyclopentyl, b0.5 200-31°. Cf. C.A. 50, 5756e, 8735a.

=> d his

(FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 16:57:23 ON 07 JUL 2004

```
L1          1629 S HOPKINS-A?/AU
L2         27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
L3             8 S  L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4             4 DUP REM L3 (4 DUPLICATES REMOVED)
L5           339 S RANDOM (5A) L2
L6             0 S  L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L7             2 S L5 AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L8          2398 S SELF-ANNEAL? OR SELF-PRIM?
L9             0 S L8 AND L5
L10            1 S L8 AND L2
L11            1 S L1 AND L2
L12          133 S L2 AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L13           83 DUP REM L12 (50 DUPLICATES REMOVED)
L14            4 S L13 AND RANDOM
L15            0 S L13 AND L8
L16         1783 S (6-MER OR 7-MER OR 8-MER)
L17            0 S L16 (8A)  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L18           11 S L16   AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L19           11 DUP REM L18 (0 DUPLICATES REMOVED)
```

=> end
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	153.84	154.05

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-4.41	-4.41

STN INTERNATIONAL LOGOFF AT 17:06:46 ON 07 JUL 2004

L2 27276 (HEXAMER OR HEPTAMER OR OCTAMER)

=> s l2 (5a) (dry or dried or freeze-dried or lyophili?)
L3 8 L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 4 DUP REM L3 (4 DUPLICATES REMOVED)

=> d ibib abs l4 1-4

L4 ANSWER 1 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-00074 BIOTECHDS
TITLE: Constructing nucleic acid array, by determining layer maps from nucleic acid sequences, indicating discrete addresses for nucleic acid subunits and, coupling transferred subunits to reactive terminus of array;
DNA array construction containing DNA probe array with application in gene expression analysis, DNA polymorphism analysis, DNA biosensor construction, etc.
AUTHOR: HUANG T
PATENT ASSIGNEE: HUANG T
PATENT INFO: US 2002168669 14 Nov 2002
APPLICATION INFO: US 2002-107556 26 Mar 2002
PRIORITY INFO: US 2002-107556 26 Mar 2002; US 2001-279004 26 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-810642 [76]
AN 2004-00074 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Constructing (M1) nucleic acid array, comprising determining layer maps from nucleic acid sequences corresponding to a particular register and indicating discrete addresses at which nucleic acid subunits are to be coupled, directing several applicator units that are supplied with a different nucleic acid subunit, and coupling the transferred nucleic acid subunits to reactive terminus of form an array of nucleic acids, is new.
DETAILED DESCRIPTION - Constructing (M1) a nucleic acid array, comprising: (a) determining several layer maps from a set of nucleic acid sequences, each layer map corresponding to a particular register and indicating discrete addresses at which corresponding nucleic acid subunits are to be coupled , for each register, (b) directing several applicator units that each are supplied with a different nucleic acid subunit to each transfer the respective nucleic acid subunit to a substrate at the discrete addresses indicated by the layer map for the respective nucleic acid subunit; and (c) coupling the transferred nucleic acid subunits to a reactive terminus of form an array of nucleic acids. INDEPENDENT CLAIMS are also included for: (1) synthesizing (M2) nucleic acids, comprising repeating for each of four nucleotide bases, adenine, guanine, cytosine, and thymidine: (a) triboelectrically charging particles of the respective nucleotide bases, the bases including a protecting group; (b) selectively irradiating a photoreceptor to generate a patterned region with defined electrostatic charge; (c) contacting the charged nucleotide particles to the photoreceptor to attach the particles to the photoreceptor in the patterned region;(d) transferring the nucleotide particles from the photoreceptor to a surface: coupling the transferred particles to terminal groups on a solid support; and (e) repeating (a)-(d) to produce several different nucleic acid sequences on the solid support; and (2) a method (M3) comprising: (a) combining toner particles less than 50 microns diameter and charged carrier beads to generate a developer particle complexes, the toner particles including a compound having a reactive group and a protecting group; (b) loading the toner particles from the developer particles complexes in a loading zone onto a surface region of a photoreceptor, the surface region being selectively charged by illumination; (c) positioning the photoreceptor to

display the surface region from the loading zone into proximity to or contact with a substrate; and (d) transferring the protected chemical compound to the substrate.

WIDER DISCLOSURE - (1) an apparatus for depositing chemical compounds on a substrate; (2) a toner composition that includes chargeable particles of diameter less than 100, 50, 20, 18, 15, 12, 10, 9, 8, 7 or 6 micro-m.

BIOTECHNOLOGY - Preferred Method: In (I), the reactive terminus is on the surface of the substrate or on the surface of the second substrate, and the nucleic acid subunits are transferred from the first substrate to the second substrate. The nucleic acid subunits are supplied as particles of less than 50 micro-m diameter. The applicator unit comprises a photoreceptor, a light unit that selectively alters the charge at discrete positions on the photoreceptor, and an agitator that agitates a toner composition comprised of the nucleic acid subunit particles and carrier beads. The applicator units comprise a common belt that rotates between the applicator sub units, and the belt hat rotates between the applicator units, and the belt includes a photoreceptive surface. In (I), the directing comprises sending the layer map for the register to each of the applicator units or generating several of image maps for the register, the image maps indicating discrete addresses at which a given nucleic acid subunit is to be coupled, and sending each of the image maps of several to the applicator unit that is supplied with the corresponding nucleic acid subunit. Preferably, the image maps are pixilated. The determining is effected by a processor. The processor is in signal communication with each of the applicator units. The first substrate is flexible. The surface is the surface of an interim substrate and involves transferring the particles from the surface of the interim substrate to the solid support, where a different interim substrate is used for each repetition of repeating and coupling step. The coupling comprises contacting the particles to an activator compound. The activator compound is chosen from tetrazole 5-(p-nitrophenyl)-1H-tetrazole, 5-ethylthio-1H-tetrazole, 4,5-dichloroimidazole, benzimidazolium triflate, or 4,5 dicyano-imidazole. The cycle of triboelectrically charging particles step, irradiating step, coupling and transferring step comprises conveying the substrate between each of four applicator units, each applicator unit comprises a photoreceptor and a source of the respective nucleotide particles. The substrate comprises paper, Mylar, cellulose polyvinyl chloride, and/or polycarbonate. In (M3), the compound is monomeric subunit of a biopolymer. The compound is a nucleotide that includes a protecting group. The nucleotide compound also includes a phosphorous activating group. (M3) further comprises contacting a solvent containing an activator compound to the substrate, where the activator compound couples the reactive group of the protected chemical compound to an immobilized group on the substrate. The transferring comprises generating an electric field.

USE - (I) is useful for multiplex analysis of both gene expression and genetic polymorphisms, screening ligands for a target compound, drug discovery screens, sensors e.g. an environmental sensor or as a diagnostic toll, e.g. to detect the presence of particular antigens, to print oligonucleotide image patterns.

ADVANTAGE - The method of dispensing the nucleic acid subunits as a dry composition which are chemically stable.

EXAMPLE - A glass slide, precoated with polyethylene glycol was spotted with a solution of 5'-dimethoxytrityl-3'-((2-cyanoethyl)-N,N-diisopropyl))-phosphoramidite of 2'deoxynucleoside. The spotting was performed on a Cartesian MicroSyS (RTM) SQ 4000 System. This device uses a solenoid to deposit solutions of phosphoramidite nucleotides. After spotting, the slide was heated at 80 degreesC for 5 minutes to evaporate the solution and form a dry film of the phophoramidite nucleotide. The slide was subsequently transferred to a glove box purged with nitrogen gas. The phosphoramidite derivative of the nucleoside was coupled to the polyethylene glycol linker on the glass slide as follows. The glass slide was sprayed with an aerosol of 0.4 M tetrazole solution in an organic solvent through a spray head for 10 seconds. The reaction was continued

for 2 minutes at and the glass slide was thoroughly washed in acetonitrile. The unreacted functional groups on the slide were capped with a solution of 1.1 M 1-methylimidaole, 0.66 M acetic anhydride, and 0.54 M 2,6 lutidine in tetrahydrofuran for 2 minutes. The slide was then removed from glove box and washed twice with acetonitrile. The phosphate trimester was oxidized with 50 mM iodine in a mixture of tetrahydrofuran-pyridine-water (93:5:2) for 1 minute. After washing with acetonitrile twice, the dimethoxytrityl protecting groups of the nucleotides were removed by treating the slide with 2 % dichloroacetic acid in methylene chloride for 1 minute. The slide was then washed with acetonitrile twice and dried at 80 degreesC for 5 minutes. The slide was subsequently plate on the Cartesian system for another cycle of spotting. These processes is repeated to synthesize the numerous different DNA sequences on the array. A 4x6 array with 1.5 mm spacings was constructed using 20 micro-l spots as above. The protecting groups were removed by treating the slide with a mixture of ethylene diamine:ethanol (1:1) for 2 hours at ambient temperature. The slide was then washed with ethanol, de-ionized water, acetone, and **dried** in air. Two different **octamer** nucleic acid sequences that differ by only a single nucleotide were synthesized on the array. The array was then tested by hybridization with a Cy3 dye labeled probe that was exactly complementary to one of the two octamer sequences. The probe was hybridized to the array at 25 degreesC for 3 hours in a humidity chamber. The slide was washed with 5XSSPE buffer for 1 minute, air dried, and scanned for Cy3 signals on a GSI Scan Array 4000 system. Addresses which have nucleic acids complementary to the probe, gave bright fluorescent signals indicating a significant hybridization between the synthesized oligonucleotides and the probe.(38 pages)

L4 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1999-05897 BIOTECHDS
TITLE: New labeling composition comprising a random mixture of 6-8 oligonucleotides;
DNA probe label addition
AUTHOR: Hopkins A
PATENT ASSIGNEE: Nycomed; Amersham
LOCATION: Little Chalfont, UK.
PATENT INFO: WO 9910531 4 Mar 1999
APPLICATION INFO: WO 1998-GB2550 21 Aug 1998
PRIORITY INFO: GB 1997-17972 22 Aug 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-190634 [16]
AN 1999-05897 BIOTECHDS
AB A labeling composition (I) comprising a random mixture of oligonucleotides which are 6-mers to 8-mers is claimed. Also claimed is preparation of labeled probes for a nucleic acid (NA) template with (I) under chain extension conditions. (I) is used for labeling NAs by a random prime method. In an example 25 ng labeling reactions were performed using a Megaprime Labeling Kit RPN 1606 or labeled probes from **dried** nonamer or **hexamer** labeling reactions. Southern blots were hybridized for 2 hr at 65 deg with the labeled probe under standard conditions and then washed in 2 x SSC, 0.1% SDS, 20 min at RT, followed by 2 washes in 0.5 x SSC, 0.1% SDS for 5 min at 65 deg. The dried blots were detected on X-ray film with 2 intensifying screens and placed into a -70 deg freezer for 11 hr. After the film was developed using a film processor it was scanned by a densitometer, then the results were analyzed by ImageQuant software. The hexamers used in a dried labeling reaction formed labeled probes, which gave a much stronger band intensity than when nonamers were used, not only when tested initially after 1 wk, but even after an extended period of storage for 37 wk at RT. (17pp)

L4 ANSWER 3 OF 4 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 93:500021 SCISEARCH

THE GENUINE ARTICLE: LR168
TITLE: NATURAL OCCURRENCE OF DENATURED PHYCOERYTHRIN DURING PORPHYRA CULTIVATION
AUTHOR: AMANO H (Reprint); NODA H
CORPORATE SOURCE: MIE UNIV, FAC BIORESOURCES, MARINE BIOCHEM LAB, TSU, MIE 514, JAPAN (Reprint)
COUNTRY OF AUTHOR: JAPAN
SOURCE: HYDROBIOLOGIA, (18 JUN 1993) Vol. 261, pp. 535-539. ISSN: 0018-8158.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 11

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Dried Nori (Porphyra spp.) normally turns greenish when toasted and this color usually remains for many days when moistened with vinegar in the Japanese food 'Sushi'. However, for some lots of toasted Nori, this color changes to an undesirable reddish-brown one within a few hours in vinegar. A clear difference was noticed by spectrophotometry between the abnormally and normally colored toasted Nori. The former exhibited absorption maxima at around 490 and 530-600 nm while these were absent in the spectrum of the latter. The responsible pigment for the abnormal coloration was purified by ammonium sulfate fractionation and preparative flat-bed isoelectric focusing. On the basis of pI, molecular weight and the visible absorption, fluorescence and circular dichroism spectra, the pigment was identified as monomeric phycoerythrin. Dried Nori contained three times as much monomer as **hexamer.** From field cultivation tests, **dried** Nori produced large amounts of monomeric phycoerythrin when the fronds on frozen nursery-nets were damaged.

L4 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 80051233 MEDLINE
DOCUMENT NUMBER: PubMed ID: 228005
TITLE: An electron paramagnetic resonance study of native and modified **freeze-dried** cupric insulin **hexamer.**
AUTHOR: Evans J C; Morgan P H; Mahbouba M; Smith H J
SOURCE: Journal of inorganic biochemistry, (1979 Oct) 11 (2) 129-37.
Journal code: 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198001
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19970203
Entered Medline: 19800124

=> d his

(FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 16:57:23 ON 07 JUL 2004
L1 1629 S HOPKINS-A?/AU
L2 27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
L3 8 S L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4 4 DUP REM L3 (4 DUPLICATES REMOVED)

=> s random (5a) l2
L5 339 RANDOM (5A) L2

=> s l5 (8a) (dry or dried or freeze-dried or lyophili?)

```
L6            0 L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> s l5 and  (dry or dried or freeze-dried or lyophili?)
L7            2 L5 AND (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> d ibib abs l7 1-2

L7    ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-10944  BIOTECHDS
TITLE:            Identifying gene expression changes within bacterial species,
                  useful for measuring gene expression profiles of prokaryotic
                  organisms, by using a comprehensive microarray synthesized
                  from DNA comprised in a bacterial species;
                     involving DNA expression, DNA microarray and DNA probe for
                     use in drug screening
AUTHOR:           LAROSSA R; WEI L
PATENT ASSIGNEE:  DU PONT DE NEMOURS and CO E I
PATENT INFO:      WO 2001029261 26 Apr 2001
APPLICATION INFO: WO 1999-US28352 15 Oct 1999
PRIORITY INFO:    US 1999-159898 15 Oct 1999
DOCUMENT TYPE:    Patent
LANGUAGE:         English
OTHER SOURCE:     WPI: 2002-226121 [28]
AN    2002-10944  BIOTECHDS
AB    DERWENT ABSTRACT:
      NOVELTY - Identifying gene expression changes within a bacterial species,
      comprising using a high-density microarray prepared with a comprehensive
      collection of open reading frames of a genome, is new.
           DETAILED DESCRIPTION - Identifying gene expression changes within a
      bacterial species, comprising: (a) providing a comprehensive micro-array
      synthesized from DNA comprised in a bacterial species; (b) generating a
      first set of labeled probes or fluorescent cDNA from bacterial RNA
      isolated from the bacterial species of (a); (c) hybridizing the first set
      of labeled probes or fluorescent cDNA to the comprehensive microarray to
      generate a detectable signal from the labeled probe; (d) measuring the
      signal generated in (c); (e) subjecting the bacterial species of (a) to a
      gene expression altering condition to alter the gene expression profile
      to produce a modified bacterial species; (f) generating a second set of
      labeled probes or fluorescent cDNA from bacterial RNA isolated from the
      modified bacterial species of (e); (g) hybridizing the second set of
      labeled probes or fluorescent cDNA to the comprehensive microarray to
      generate a detectable signal from the labeled probe or fluorescent cDNA;
      (h) measuring the signal generated in (g); and (i) comparing the signals
      generated from the first hybridization to that of the second
      hybridization; INDEPENDENT CLAIMS are included for the following: (1)
      identifying gene expression changes within a genome, comprising: (a)
      providing a comprehensive micro-array synthesized from DNA comprised in a
      prokaryotic or eukaryotic species; (b) generating a control set of
      fluorescent cDNA from total or polyadenylated RNA isolated from the
      species of (a), where the fluorescent cDNA comprises at least one first
      fluorescent label and at least one different second fluorescent label;
      (c) mixing the control set of fluorescent cDNA labeled with at a first
      label with the control set of fluorescent cDNA labeled with a second
      first label to form a dual labeled control cDNA; (d) hybridizing the dual
      labeled control set of fluorescent cDNA to the comprehensive micro-array,
      where hybridization results in a detectable signal generated from the
      fluorescent cDNA; (e) measuring the signal generated by the hybridization
      of the dual labeled control set of fluorescent cDNA to the comprehensive
      micro-array of (c); (f) subjecting the prokaryote or eukaryote to a gene
      expression altering condition to produce a modified prokaryote or
      eukaryote; (g) generating an experimental set of fluorescent cDNA from
      total or polyadenylated RNA isolated from the modified prokaryote or
      eukaryote in (e), where the fluorescent cDNA comprises the first
      fluorescent label and the different second fluorescent label; (h) mixing
      the experimental sets of fluorescent cDNAs labeled with the first and
```

second labels to form a dual labeled experimental cDNA; (i) hybridizing the experimental set of fluorescent cDNA in (h) to the comprehensive micro-array, to generate a detectable signal from the fluorescent cDNA; (j) measuring the signal generated in (i); and (k) comparing signal generated from the dual labeled control hybridization with the dual labeled experimental hybridization; and (2) a method for quantifying the amount of protein specifying RNA contained within a genome.

BIOTECHNOLOGY - Preferred Method: The bacterial species is selected from enteric bacteria, Bacillus, Acinetobacter, Streptomyces, Methylobacter, Pseudomonas, Rhodobacter and Synechocystis. The signal-generating label consists of fluorescent moieties, chemiluminescent moieties, particles, enzymes, or radioactive tags, preferably a fluorescent moiety selected from cy3 and cy5. The comprehensive microarray contains at least 75 % of all open reading frames in the bacterial species, preferably 2000-6000 open reading frames. The gene expression altering condition consists of a condition altering the genotype of the bacterial species, a condition altering the growth of the bacterial species, exposure to mutagens, antibiotics, ultraviolet (UV) light, gamma-rays, x-rays, phage, macrophages, organic chemicals, inorganic chemicals, environmental pollutants, heavy metals, changes in temperature, changes in pH, conditions producing oxidative damage, DNA damage, anaerobiosis, depletion or addition of nutrients, addition of a growth inhibitor, and desiccation. In identifying gene expression changes, the first and second fluorescent label is independently selected from cy3 and cy5. The prokaryotic or eukaryotic genome is comprised within an organism selected an enteric bacteria, Bacillus, Acinetobacter, Streptomyces, Methylobacter, Pseudomonas, cyanobacteria, yeasts, filamentous fungi, plant cells and animal cells. The yeast is Saccharomyces, Zygosaccharomyces, Kluyveromyces, Candida, Hansenula, Debaryomyces, Mucor, Pichia or Torulopsis. The cyanobacteria are selected Rhodobacter and Synechocystis. The filamentous fungi may be Aspergillus or Arthrobotrys. Identifying gene expression changes within a bacterial species also comprises quantifying the amount of protein specifying RNA contained within a genome Quantifying the amount of protein specifying RNA contained within a genome comprises: (a) providing a comprehensive micro-array comprising a multiple open reading frames synthesized from genomic DNA comprised in a prokaryotic or eukaryotic organism; (b) generating a set of fluorescent cDNA from total or poly-adenylated RNA isolated from the prokaryotic or eukaryotic organism in (a); (c) generating a set of fluorescent DNA from genomic DNA isolated from the prokaryotic or eukaryotic organism; (d) hybridizing the fluorescent cDNA in (b) to the comprehensive micro-array in (a), to generate a first fluorescent signal from the fluorescent cDNA for each open reading frame; (e) hybridizing the fluorescent DNA to the comprehensive micro-array, to generate a fluorescent signal from the fluorescent DNA for each open reading frame; and (f) dividing, for each open reading frame, the first fluorescent signal into the second fluorescent signal to provide a quantified measure of the amount of protein specifying RNA for each open reading frame.Alternatively, (a) includes providing a comprehensive micro-array comprising a multiple genes synthesized from genomic DNA comprised in a prokaryotic or eukaryotic organism.

USE - The method is useful for measuring the changes in gene expression profiles of prokaryotic organisms, and the levels of protein specifying RNA in prokaryotic and/or eukaryotic organisms, and for comparing the gene expression patterns of 2 samples differing in one variable. The method is useful in screening discovery compounds by comparing their gene expression profile to a known compound that affects the desirable target gene products, where gene expression profiles are good indicators of genotypic alterations among strains. The method allows the discovery of complementary target inhibitors in combination drug therapy and may be used as a modeling system to test perturbations in process conditions to determine the conditions for the high yield of desired production in various bioprocesses and biotransformations.

ADVANTAGE - The new method allows the monitoring the comprehensive

responses of a preponderance of individual genes in the genome of an organism in reliable, useful manner, and provides a way to measure the comprehensive gene expression profile analysis of the organism.

EXAMPLE - Escherichia coli was cultured in minimal medium supplemented with 0.4 % glucose at 37 degrees C. Overnight culture was diluted in fresh medium and aerated by shaking. An isopropyl-beta--thiogalactopyranoside (IPTG) induction was performed to examine the specificity with which it effects gene expression. To one portion of the culture was added IPTG, and the untreated sample served as control RNA was isolated from the cells, and from the total RNA, fluorescent DNA was synthesized. Genomic DNA isolated from strain MG1655 was nebulized to 2 kbase pair fragments. Three micrograms of this DNA was mixed with **random hexamer** primers. DNA was denatured by heating at 94 degrees C prior to annealing on ice. Fluorescent copying of the genomic DNA was accomplished using the Klenow fragment of DNA polymerase I. Spotted Slides were placed in isopropanol for 10 minutes, boiled in water for 5 minutes, and **dried** by passage of ultra-clean N2 gas prior to pre-hybridization. Slides were incubated in pre-hybridization solution, rinsed in water, then isopropanol, and applied with probe containing hybridization solution. Hybridization to each slide was quantified with confocal laser microscope whose photomultiplier tube was set to 700 volts and 800 volts for obtaining Cy3 and Cy5 signals, respectively. Fluorescent intensity associated with each spotted gene was reduced by subtracting the fluorescence of an adjoining non-spotted region of the slide. No results were given.(81 pages)

L7 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1993-06701 BIOTECHDS
TITLE: High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast S. pombe;
Schizosaccharomyces pombe P1 gene bank construction and cosmid bank construction using a high density filter and yeast artificial chromosome for application in mapping
AUTHOR: Hoheisel J D; Maier E; Mott R; McCarthy L; Grigoriev A V; Schalkwyk L C
LOCATION: Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.
SOURCE: Cell; (1993) 73, 1, 109-20
CODEN: CELLB5
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1993-06701 BIOTECHDS
AB A P1 gene bank of 17-fold coverage and a cosmid bank of 8 genome equivalents gridded on high density filters were constructed from Schizosaccharomyces pombe 972h-. Yeast artificial chromosome clones covering the entire genome were used to subdivide the banks. Cosmid, P1 and marker DNAs were prepared by an alkaline lysis method. Probe DNA was radioactively labeled by **random hexamer** priming or, in the case of cosmid pools, by primer extension reaction. The bank filters were prehybridized in 0.5 M sodium phosphate (pH 7.2), 7% SDS, 1 MM EDTA and 0.1 mg/ml yeast tRNA. Hybridization was performed in the buffer at 65 deg overnight. The filters were rinsed and then washed by rocking slowly in a water bath. The filters blotted **dry** and film was exposed for 2 hr overnight at -70 deg. The probe was stripped off the filters and the filters produced reasonable results after 30 cycles. The high resolution clone map was aligned to the genetic map and the physical NotI and yeast artificial chromosome maps. (35 ref)

=> d his

(FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 16:57:23 ON 07 JUL 2004

```
L1          1629 S HOPKINS-A?/AU
L2         27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
L3             8 S  L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4             4 DUP REM L3 (4 DUPLICATES REMOVED)
L5           339 S RANDOM (5A) L2
L6             0 S  L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L7             2 S L5 AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> s self-anneal? or self-prim?
L8          2398 SELF-ANNEAL? OR SELF-PRIM?

=> s l8 and l5
L9             0 L8 AND L5

=> s l8 and l2
L10            1 L8 AND L2

=> d all

L10   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN    1993-00028  BIOTECHDS
TI    Simplified methods for construction, assessment and rapid screening of
      peptide libraries in bacteriophage;
         phage fd pIII protein peptide library construction in phage vector
AU    Christian R B; Zuckermann R N; Kerr J M; Wang L; *Malcolm B A
CS    Chiron
LO    Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G
      2H7, Canada.
SO    J.Mol.Biol.; (1992) 227, 3, 711-18
      CODEN: JMOBAK
DT    Journal
LA    English
AB    An efficient strategy has been devised for the construction of diverse,
      large and complex peptide libraries in phage vectors using a self
      complementary, self-priming oligonucleotide which
      increases the efficiency of both the annealing and priming reactions.
      This strategy was used to generate a library of 400,000,000 random
      decapeptide inserts in the pIII protein of phage fd.  The library was
      large enough to ensure that a complete set of hexamer peptide
      sequences were represented in the 5 possible hexamer frames
      within the decapeptide sequence.  A novel method for evaluating the
      genetic diversity of phage libraries based on colony hybridization with
      partially degenerate oligonucleotides was developed.  The decapeptide
      library was affinity-selected with a previously characterized monoclonal
      antibody specific for the V3 loop of the HIV virus-1 gp120 protein.  This
      immunological screening technique represented an improvement in phage
      library screening, as it allowed the rapid, direct identification of
      binding phage, and is therefore a powerful tool for choosing appropriate
      peptide ligands as candidates for further investigation.  (21 ref)
CC    A GENETIC ENGINEERING AND FERMENTATION; A1 Nucleic Acid Technology
CT    PHAGE FD PIII PROTEIN DECA-PEPTIDE LIBRARY CONSTRUCTION IN PHAGE VECTOR,
      GENETIC DIVERSITY, IMMUNOLOGICAL SCREENING, POT. PEPTIDE LIGAND
      IDENTIFICATION .


=> d his

     (FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 16:57:23 ON 07 JUL 2004
L1          1629 S HOPKINS-A?/AU
L2         27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
L3             8 S  L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4             4 DUP REM L3 (4 DUPLICATES REMOVED)
```

```
L5          339 S RANDOM (5A) L2
L6            0 S  L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L7            2 S L5 AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L8         2398 S SELF-ANNEAL? OR SELF-PRIM?
L9            0 S L8 AND L5
L10           1 S L8 AND L2

=> s ;1 and l2
ENTER LOGIC EXPRESSION, QUERY NAME, OR (END):end
SEARCH ENDED BY USER


1 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l1 and l2
L11           1 L1 AND L2

=> d all

L11   ANSWER 1 OF 1  BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AN    1999-05897  BIOTECHDS
TI    New labeling composition comprising a random mixture of 6-8
      oligonucleotides;
         DNA probe label addition
AU    Hopkins A
PA    Nycomed; Amersham
LO    Little Chalfont, UK.
PI    WO 9910531 4 Mar 1999
AI    WO 1998-GB2550 21 Aug 1998
PRAI  GB 1997-17972 22 Aug 1997
DT    Patent
LA    English
OS    WPI: 1999-190634 [16]
AB    A labeling composition (I) comprising a random mixture of
      oligonucleotides which are 6-mers to 8-mers is claimed.  Also claimed is
      preparation of labeled probes for a nucleic acid (NA) template with (I)
      under chain extension conditions.  (I) is used for labeling NAs by a
      random prime method.  In an example 25 ng labeling reactions were
      performed using a Megaprime Labeling Kit RPN 1606 or labeled probes from
      dried nonamer or hexamer labeling reactions.  Southern blots
      were hybridized for 2 hr at 65 deg with the labeled probe under standard
      conditions and then washed in 2 x SSC, 0.1% SDS, 20 min at RT, followed
      by 2 washes in 0.5 x SSC, 0.1% SDS for 5 min at 65 deg.  The dried blots
      were detected on X-ray film with 2 intensifying screens and placed into a
      -70 deg freezer for 11 hr.  After the film was developed using a film
      processor it was scanned by a densitometer, then the results were
      analyzed by ImageQuant software.  The hexamers used in a dried labeling
      reaction formed labeled probes, which gave a much stronger band intensity
      than when nonamers were used, not only when tested initially after 1 wk,
      but even after an extended period of storage for 37 wk at RT.  (17pp)
CC    A GENETIC ENGINEERING AND FERMENTATION; A1 Nucleic Acid Technology
CT    OLIGONUCLEOTIDE DNA PROBE LABEL HYBRIDIZATION (VOL.18, NO.11)


=> d his

     (FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 16:57:23 ON 07 JUL 2004
L1         1629 S HOPKINS-A?/AU
L2        27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
```

```
L3             8 S  L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4             4 DUP REM L3 (4 DUPLICATES REMOVED)
L5           339 S RANDOM (5A) L2
L6             0 S  L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L7             2 S L5 AND  (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L8          2398 S SELF-ANNEAL? OR SELF-PRIM?
L9             0 S L8 AND L5
L10            1 S L8 AND L2
L11            1 S L1 AND L2

=> s l2 and  (dry or dried or freeze-dried or lyophili?)
L12          133 L2 AND (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13           83 DUP REM L12 (50 DUPLICATES REMOVED)

=> s l13 and random
L14            4 L13 AND RANDOM

=> d ibib abs l14 1-4

L14  ANSWER 1 OF 4      MEDLINE on STN
ACCESSION NUMBER:    2003565573      IN-PROCESS
DOCUMENT NUMBER:     PubMed ID: 14645090
TITLE:               Conformation of reconstituted mononucleosomes and effect of
                     linker histone H1 binding studied by scanning force
                     microscopy.
AUTHOR:              Kepert Jochen Felix; Toth Katalin Fejes; Caudron Maiwen;
                     Mucke Norbert; Langowski Jorg; Rippe Karsten
CORPORATE SOURCE:    Kirchhoff-Institut fur Physik, AG Molekulare Biophysik
                     (F15), Ruprecht-Karls-Universitat Heidelberg, D-69120
                     Heidelberg, Germany.
SOURCE:              Biophysical journal, (2003 Dec) 85 (6) 4012-22.
                     Journal code: 0370626. ISSN: 0006-3495.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE:          Entered STN: 20031216
                     Last Updated on STN: 20031225
```

AB The conformation of mononucleosome complexes reconstituted with recombinant core histones on a 614-basepair-long DNA fragment containing the Xenopus borealis 5S rRNA nucleosome positioning sequence was studied by scanning/atomic force microscopy in the absence or presence of linker histone H1. Imaging without prior fixation was conducted with air-**dried** samples and with mononucleosomes that were injected directly into the scanning force microscopy fluid cell and visualized in buffer. From a quantitative analysis of approximately 1,700 complexes, the following results were obtained: i), In the absence of H1, a preferred location of the nucleosome at the X. borealis 5S rRNA sequence in the center of the DNA was detected. From the distribution of nucleosome positions, an energy difference of binding to the 5S rRNA sequence of DeltaDeltaG approximately 3 kcal mol(-1) as compared to a **random** sequence was estimated. Upon addition of H1, a significantly reduced preference of nucleosome binding to this sequence was observed. ii), The measured entry-exit angles of the DNA at the nucleosome in the absence of H1 showed two maxima at 81 +/- 29 degrees and 136 +/- 18 degrees (air-**dried** samples), and 78 +/- 25 degrees and 137 +/- 25 degrees (samples imaged in buffer solution). In the presence of H1, the species with the smaller entry-exit angle was stabilized, yielding average values of 88 +/- 34 degrees for complexes in air and 85 +/- 10 degrees in buffer solution. iii), The apparent contour length of the nucleosome complexes was shortened by 34 +/- 13 nm as compared to the free DNA due to wrapping of the DNA around the histone **octamer** complex. Considering an

11 nm diameter of the nucleosome core complex, this corresponds to a total of 145 +/- 34 basepairs that are wound around the nucleosome.

L14 ANSWER 2 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-10944 BIOTECHDS
TITLE: Identifying gene expression changes within bacterial species, useful for measuring gene expression profiles of prokaryotic organisms, by using a comprehensive microarray synthesized from DNA comprised in a bacterial species; involving DNA expression, DNA microarray and DNA probe for use in drug screening
AUTHOR: LAROSSA R; WEI L
PATENT ASSIGNEE: DU PONT DE NEMOURS and CO E I
PATENT INFO: WO 2001029261 26 Apr 2001
APPLICATION INFO: WO 1999-US28352 15 Oct 1999
PRIORITY INFO: US 1999-159898 15 Oct 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-226121 [28]
AN 2002-10944 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying gene expression changes within a bacterial species, comprising using a high-density microarray prepared with a comprehensive collection of open reading frames of a genome, is new.

DETAILED DESCRIPTION - Identifying gene expression changes within a bacterial species, comprising: (a) providing a comprehensive micro-array synthesized from DNA comprised in a bacterial species; (b) generating a first set of labeled probes or fluorescent cDNA from bacterial RNA isolated from the bacterial species of (a); (c) hybridizing the first set of labeled probes or fluorescent cDNA to the comprehensive microarray to generate a detectable signal from the labeled probe; (d) measuring the signal generated in (c); (e) subjecting the bacterial species of (a) to a gene expression altering condition to alter the gene expression profile to produce a modified bacterial species; (f) generating a second set of labeled probes or fluorescent cDNA from bacterial RNA isolated from the modified bacterial species of (e); (g) hybridizing the second set of labeled probes or fluorescent cDNA to the comprehensive microarray to generate a detectable signal from the labeled probe or fluorescent cDNA; (h) measuring the signal generated in (g); and (i) comparing the signals generated from the first hybridization to that of the second hybridization; INDEPENDENT CLAIMS are included for the following: (1) identifying gene expression changes within a genome, comprising: (a) providing a comprehensive micro-array synthesized from DNA comprised in a prokaryotic or eukaryotic species; (b) generating a control set of fluorescent cDNA from total or polyadenylated RNA isolated from the species of (a), where the fluorescent cDNA comprises at least one first fluorescent label and at least one different second fluorescent label; (c) mixing the control set of fluorescent cDNA labeled with at a first label with the control set of fluorescent cDNA labeled with a second first label to form a dual labeled control cDNA; (d) hybridizing the dual labeled control set of fluorescent cDNA to the comprehensive micro-array, where hybridization results in a detectable signal generated from the fluorescent cDNA; (e) measuring the signal generated by the hybridization of the dual labeled control set of fluorescent cDNA to the comprehensive micro-array of (c); (f) subjecting the prokaryote or eukaryote to a gene expression altering condition to produce a modified prokaryote or eukaryote; (g) generating an experimental set of fluorescent cDNA from total or polyadenylated RNA isolated from the modified prokaryote or eukaryote in (e), where the fluorescent cDNA comprises the first fluorescent label and the different second fluorescent label; (h) mixing the experimental sets of fluorescent cDNAs labeled with the first and second labels to form a dual labeled experimental cDNA; (i) hybridizing the experimental set of fluorescent cDNA in (h) to the comprehensive micro-array, to generate a detectable signal from the fluorescent cDNA; (j) measuring the signal generated in (i); and (k) comparing signal

generated from the dual labeled control hybridization with the dual labeled experimental hybridization; and (2) a method for quantifying the amount of protein specifying RNA contained within a genome.

BIOTECHNOLOGY - Preferred Method: The bacterial species is selected from enteric bacteria, Bacillus, Acinetobacter, Streptomyces, Methylobacter, Pseudomonas, Rhodobacter and Synechocystis. The signal-generating label consists of fluorescent moieties, chemiluminescent moieties, particles, enzymes, or radioactive tags, preferably a fluorescent moiety selected from cy3 and cy5. The comprehensive microarray contains at least 75 % of all open reading frames in the bacterial species, preferably 2000-6000 open reading frames. The gene expression altering condition consists of a condition altering the genotype of the bacterial species, a condition altering the growth of the bacterial species, exposure to mutagens, antibiotics, ultraviolet (UV) light, gamma-rays, x-rays, phage, macrophages, organic chemicals, inorganic chemicals, environmental pollutants, heavy metals, changes in temperature, changes in pH, conditions producing oxidative damage, DNA damage, anaerobiosis, depletion or addition of nutrients, addition of a growth inhibitor, and desiccation. In identifying gene expression changes, the first and second fluorescent label is independently selected from cy3 and cy5. The prokaryotic or eukaryotic genome is comprised within an organism selected an enteric bacteria, Bacillus, Acinetobacter, Streptomyces, Methylobacter, Pseudomonas, cyanobacteria, yeasts, filamentous fungi, plant cells and animal cells. The yeast is Saccharomyces, Zygosaccharomyces, Kluyveromyces, Candida, Hansenula, Debaryomyces, Mucor, Pichia or Torulopsis. The cyanobacteria are selected Rhodobacter and Synechocystis. The filamentous fungi may be Aspergillus or Arthrobotrys. Identifying gene expression changes within a bacterial species also comprises quantifying the amount of protein specifying RNA contained within a genome Quantifying the amount of protein specifying RNA contained within a genome comprises: (a) providing a comprehensive micro-array comprising a multiple open reading frames synthesized from genomic DNA comprised in a prokaryotic or eukaryotic organism; (b) generating a set of fluorescent cDNA from total or poly-adenylated RNA isolated from the prokaryotic or eukaryotic organism in (a); (c) generating a set of fluorescent DNA from genomic DNA isolated from the prokaryotic or eukaryotic organism; (d) hybridizing the fluorescent cDNA in (b) to the comprehensive micro-array in (a), to generate a first fluorescent signal from the fluorescent cDNA for each open reading frame; (e) hybridizing the fluorescent DNA to the comprehensive micro-array, to generate a fluorescent signal from the fluorescent DNA for each open reading frame; and (f) dividing, for each open reading frame, the first fluorescent signal into the second fluorescent signal to provide a quantified measure of the amount of protein specifying RNA for each open reading frame.Alternatively, (a) includes providing a comprehensive micro-array comprising a multiple genes synthesized from genomic DNA comprised in a prokaryotic or eukaryotic organism.

USE - The method is useful for measuring the changes in gene expression profiles of prokaryotic organisms, and the levels of protein specifying RNA in prokaryotic and/or eukaryotic organisms, and for comparing the gene expression patterns of 2 samples differing in one variable. The method is useful in screening discovery compounds by comparing their gene expression profile to a known compound that affects the desirable target gene products, where gene expression profiles are good indicators of genotypic alterations among strains. The method allows the discovery of complementary target inhibitors in combination drug therapy and may be used as a modeling system to test perturbations in process conditions to determine the conditions for the high yield of desired production in various bioprocesses and biotransformations.

ADVANTAGE - The new method allows the monitoring the comprehensive responses of a preponderance of individual genes in the genome of an organism in reliable, useful manner, and provides a way to measure the comprehensive gene expression profile analysis of the organism.

EXAMPLE - Escherichia coli was cultured in minimal medium

supplemented with 0.4 % glucose at 37 degrees C. Overnight culture was diluted in fresh medium and aerated by shaking. An isopropyl-beta--thiogalactopyranoside (IPTG) induction was performed to examine the specificity with which it effects gene expression. To one portion of the culture was added IPTG, and the untreated sample served as control RNA was isolated from the cells, and from the total RNA, fluorescent DNA was synthesized. Genomic DNA isolated from strain MG1655 was nebulized to 2 kbase pair fragments. Three micrograms of this DNA was mixed with **random hexamer** primers. DNA was denatured by heating at 94 degrees C prior to annealing on ice. Fluorescent copying of the genomic DNA was accomplished using the Klenow fragment of DNA polymerase I. Spotted Slides were placed in isopropanol for 10 minutes, boiled in water for 5 minutes, and **dried** by passage of ultra-clean N2 gas prior to pre-hybridization. Slides were incubated in pre-hybridization solution, rinsed in water, then isopropanol, and applied with probe containing hybridization solution. Hybridization to each slide was quantified with confocal laser microscope whose photomultiplier tube was set to 700 volts and 800 volts for obtaining Cy3 and Cy5 signals, respectively. Fluorescent intensity associated with each spotted gene was reduced by subtracting the fluorescence of an adjoining non-spotted region of the slide. No results were given.(81 pages)

L14 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1999-05897 BIOTECHDS
TITLE: New labeling composition comprising a **random** mixture of 6-8 oligonucleotides;
DNA probe label addition
AUTHOR: Hopkins A
PATENT ASSIGNEE: Nycomed; Amersham
LOCATION: Little Chalfont, UK.
PATENT INFO: WO 9910531 4 Mar 1999
APPLICATION INFO: WO 1998-GB2550 21 Aug 1998
PRIORITY INFO: GB 1997-17972 22 Aug 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1999-190634 [16]
AN 1999-05897 BIOTECHDS
AB A labeling composition (I) comprising a **random** mixture of oligonucleotides which are 6-mers to 8-mers is claimed. Also claimed is preparation of labeled probes for a nucleic acid (NA) template with (I) under chain extension conditions. (I) is used for labeling NAs by a **random** prime method. In an example 25 ng labeling reactions were performed using a Megaprime Labeling Kit RPN 1606 or labeled probes from **dried** nonamer or **hexamer** labeling reactions. Southern blots were hybridized for 2 hr at 65 deg with the labeled probe under standard conditions and then washed in 2 x SSC, 0.1% SDS, 20 min at RT, followed by 2 washes in 0.5 x SSC, 0.1% SDS for 5 min at 65 deg. The **dried** blots were detected on X-ray film with 2 intensifying screens and placed into a -70 deg freezer for 11 hr. After the film was developed using a film processor it was scanned by a densitometer, then the results were analyzed by ImageQuant software. The hexamers used in a **dried** labeling reaction formed labeled probes, which gave a much stronger band intensity than when nonamers were used, not only when tested initially after 1 wk, but even after an extended period of storage for 37 wk at RT. (17pp)

L14 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1993-06701 BIOTECHDS
TITLE: High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast S. pombe;
Schizosaccharomyces pombe P1 gene bank construction and cosmid bank construction using a high density filter and yeast artificial chromosome for application in mapping
AUTHOR: Hoheisel J D; Maier E; Mott R; McCarthy L; Grigoriev A V; Schalkwyk L C

LOCATION: Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.
SOURCE: Cell; (1993) 73, 1, 109-20
CODEN: CELLB5
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1993-06701 BIOTECHDS
AB A P1 gene bank of 17-fold coverage and a cosmid bank of 8 genome equivalents gridded on high density filters were constructed from Schizosaccharomyces pombe 972h-. Yeast artificial chromosome clones covering the entire genome were used to subdivide the banks. Cosmid, P1 and marker DNAs were prepared by an alkaline lysis method. Probe DNA was radioactively labeled by **random hexamer** priming or, in the case of cosmid pools, by primer extension reaction. The bank filters were prehybridized in 0.5 M sodium phosphate (pH 7.2), 7% SDS, 1 MM EDTA and 0.1 mg/ml yeast tRNA. Hybridization was performed in the buffer at 65 deg overnight. The filters were rinsed and then washed by rocking slowly in a water bath. The filters blotted **dry** and film was exposed for 2 hr overnight at -70 deg. The probe was stripped off the filters and the filters produced reasonable results after 30 cycles. The high resolution clone map was aligned to the genetic map and the physical NotI and yeast artificial chromosome maps. (35 ref)

=> d his

(FILE 'HOME' ENTERED AT 16:57:13 ON 07 JUL 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 16:57:23 ON 07 JUL 2004
L1 1629 S HOPKINS-A?/AU
L2 27276 S (HEXAMER OR HEPTAMER OR OCTAMER)
L3 8 S L2 (5A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L4 4 DUP REM L3 (4 DUPLICATES REMOVED)
L5 339 S RANDOM (5A) L2
L6 0 S L5 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L7 2 S L5 AND (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L8 2398 S SELF-ANNEAL? OR SELF-PRIM?
L9 0 S L8 AND L5
L10 1 S L8 AND L2
L11 1 S L1 AND L2
L12 133 S L2 AND (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)
L13 83 DUP REM L12 (50 DUPLICATES REMOVED)
L14 4 S L13 AND RANDOM

=> s l13 and l8
L15 0 L13 AND L8

=> s (6-mer or 7-mer or 8-mer)
L16 1783 (6-MER OR 7-MER OR 8-MER)

=> s l16 (8a) (dry or dried or freeze-dried or lyophili?)
L17 0 L16 (8A) (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> s l16 and (dry or dried or freeze-dried or lyophili?)
L18 11 L16 AND (DRY OR DRIED OR FREEZE-DRIED OR LYOPHILI?)

=> dup rem ;18
ENTER L# LIST OR (END):l18
PROCESSING COMPLETED FOR L18
L19 11 DUP REM L18 (0 DUPLICATES REMOVED)

18 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.

For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> d ibib abs l19 1-11

L19 ANSWER 1 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-19388 BIOTECHDS
TITLE: Diagnosing patient for carcinoma by administering labeled alpha-fetoprotein peptide which comprises hydrophilic analog of alpha-fetoprotein peptide and metal ion to patient, imaging labeled peptide after its localization; using gamma scintigraphy, specific photon emission computerized tomography, positron emission tomography or NMR
AUTHOR: ANDERSEN T T; BENNETT J A; JACOBSON H I; MESFIN F B
PATENT ASSIGNEE: CLF MEDICAL TECHNOLOGY ACCELERATION PROG
PATENT INFO: WO 2003044041 30 May 2003
APPLICATION INFO: WO 2002-US37291 20 Nov 2002
PRIORITY INFO: US 2002-409109 9 Sep 2002; US 2001-331841 20 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-505137 [47]
AN 2003-19388 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Diagnosing (M1) a patient, involves administering labeled alpha-fetoprotein (AFP) peptide (I) 8-20 amino acids in length, where labeled (I) comprises a hydrophilic analog of an AFP peptide and a medically useful metal ion to a patient, allowing for localization of the labeled peptide, and imaging the labeled peptide.

DETAILED DESCRIPTION - Diagnosing a patient, involves administering labeled AFP peptide (I) 8-20 amino acids in length, (I) comprising a hydrophilic analog of an AFP peptide of the sequence Glu-Met-Thr-Pro-Val-Asn-Pro-Gly (S6) and a medically useful metal ion to a patient, allowing for localization of the labeled peptide, and imaging the labeled peptide. The method optionally involves administering a labeled peptide which comprises a hydrophilic analog of AFP, having the structure Xaa1-Xaa2-Xaa3-O-Xaa4-N-Xaa5-G-Xaa6, where Xaa1 is chosen from Glu, Gln, and Asn or its acetylated or acylated derivative, Xaa2 is chosen from Met and Lys, or their analogs, Xaa3 is a structure providing steric hindrance and hydrophilicity and can be chosen from Thr and Ser, Xaa4 is chosen from Val, Ile, Leu, Thr, a beta-branched amino acid structure, and a hydrophobic amino acid structure, Xaa5 is chosen from Pro, hydroxyproline (O) and Ser, and Xaa6 is an amino acid structure which may be present or absent, or a substitution variant, peptidomimetic, retro-inverso isomer, or its salt. INDEPENDENT CLAIMS are also included for the following: (1) an AFP peptide (P1) comprising a targeting moiety comprising the amino acid sequence (S6), and a metal ion-binding domain; (2) an AFP peptide (P2) comprising a targeting moiety which comprises a hydrophilic analog of an AFP, having the sequence Xaa1-Xaa2-Xaa3-O-Xaa4-N-Xaa5-G-Xaa6; (3) an imaging agent (II) comprising a paramagnetic metal ion bound to a complex, comprises a chelator, a targeting moiety comprising an AFP peptide having a peptide sequence 8-20 amino acids long, which comprises hydrophilic analog of AFP peptide of sequence (S6), where the targeting moiety is covalently attached to the chelator which binds in at least a first coordination site of the metal ion and which is capable of interacting with a target substance such that the exchange of water in at least the first coordination site is increased; and (4) an imaging agent (III) comprising a paramagnetic metal ion capable of binding n coordination atoms, where the metal ion is bound to a chelator such that the metal ion has coordination atoms at (n-1) or (n-2) coordination sites of the metal ion, and a targeting moiety comprising an AFP peptide having a peptide sequence eight to twenty amino acids long, which comprises a hydrophilic analog of an AFP peptide of the sequence (S6), the targeting moiety covalently attached to the chelator that hinders the rapid exchange of water in the remaining coordination site or sites, where the

targeting moiety is capable of interacting with a target substance, such that the exchange of water at the remaining coordination site or sites is increased.

WIDER DISCLOSURE - Labeling AFP peptides with the metal ion, is also disclosed.

BIOTECHNOLOGY - Preferred Method: The hydrophilic analog is chosen from Glu-Lys-Thr-O-Val-Asn-O-Gly-Asn (S1), Gln-Met-Thr-Pro-Val-Asn-Pro-Gly (S2), Gln-Met-Thr-Pro-Val-Asn-Pro-Gly-Glu (S3), Glu-Met-Thr-O-Val-Asn-O-Gly (S4), Glu-Met-Thr-O-Val-Asn-O-Gly-Gln (S5), Glu-Met-Thr-Pro-Val-Asn-Pro-Gly (S6), Glu-Met-Thr-Pro-Val-Asn-Pro-Gly-Gln (S7), Glu-Met-Thr-O-Val-Asn-Pro-Gly-Gln (S8), Glu-Met-Thr-Pro-Val-Asn-O-Gly-Gln (S9) (where O is hydroxyproline). Labeled (I) further comprises a chelating agent (e.g. a bifunctional agent), where the medically useful metal ion is bound to the peptide via the chelating agent. Labeled (I) preferably comprises the sequence (S6) and further comprises a metal ion-binding domain, where the linked medically useful metal ion is bound to the peptide through the metal ion-binding domain. Labeled (I) is chosen from (R1)--(Y1)n--(R2), (R1)--(Y1--(R2)--Y1)n--(R3), and (R1)--(Y1--(R2)--Y2)n--(R3) where the metal ion-binding domain is (Y1)n, (Y1--(R2)--Y1)n, or (Y1--(R2)--Y2)n, where n 1-6, and Y1 and Y2 are amino acids comprising at least one element chosen from sulfur, nitrogen and oxygen which is available or can be made available for binding to metal ions; the peptide sequence (S6) is present in at least one member chosen from R1, R2 and R3 and further comprises an amino acid sequence containing from 5-20 amino acids, and those portions of R1, R2 and R3 not comprising the peptide sequence (S6) each comprise an amino acid sequence containing from 0-20 amino acids. The metal ion-binding domain comprises at least one amino acid chosen from cysteine, cystine, histidine, penicillamine, deacylated methionine, lysine, arginine, aspartic acid, glutamic acid and tyrosine. The metal ion-binding domain more preferably comprises at least one member chosen from (Cys)n, (Cys--(R2)--Cys)n, (Cys--(R2)--Pen)n, (His--(R2)--Cys)n, (His--(R2)--Pen)n, (His)n, and ((His--(R2)--His)n, where n is 1-6, and R2 is an amino acid sequence containing 1-20 amino acids. The imaging is chosen from gamma scintigraphy, specific photon emission computerized tomography, positron emission tomography and magnetic resonance imaging. The medically useful metal ion comprises at least one ionic element chosen from iron, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, technetium, ruthenium, palladium, silver, cadmium, indium, antimony, rhenium, osmium, iridium, platinum, gold, mercury, thallium, lead, bismuth, polonium and astatine, and comprises at least one property chosen from radioactivity, paramagnetism and superparamagnetism. (M1), preferably involves diagnosing a cell-proliferative disorder, which involves providing a medically useful metal ion-labeled (I) comprising a peptide sequence of 8-20 amino acids in length which comprises a hydrophilic analog of AFP peptide of sequence (S6) and a metal ion, administering the metal ion-labeled AFP peptide to the patient, allowing for localization of the metal ion-labeled AFP peptide, and imaging the metal ion-labeled alpha-fetoprotien peptide. Preferred Peptide: (P1) further comprises a metal ion coupled to the metal ion-binding domain. The biological-function domain comprises an amino acid sequence of 8-20 amino acids. The metal ion is radioactive and is chosen from technetium (preferably technetium-99m) and rhenium (rhenium-186 or rhenium-188). The targeting moiety comprises a hydrophilic analog of the sequence (S6), and is chosen from (S1)-(S9). Preferred Agent: In (II), the chelator is 1,4,7,10-tetraazacyclododecane-N,N', N'',N'''- tetraacetic acid (DOTA) and diethylenetriaminepentaacetic acid (DTPA), or is a polymer backbone. The paramagnetic metal ion is chosen from Gd(III), Fe(III), Mn(II), Yt(III), Cr(III) or Dy(III), preferably Gd(III). (III) comprises a Gd(III) ion bound to a chelator with 8 coordination atoms at 8 coordination sites of the Gd(III) ion, and a targeting moiety which hinders the rapid exchange of water in a 9th coordination site, where the targeting moiety is capable of interacting with a target substance, such that the exchange of water at the 9th coordination site is increased. Preferably, a Gd(III) ion is bound to a chelator with 8 coordination

atoms.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Cancer cell growth inhibitor. An **8-mer** peptide (Glu-Met-Thr-O-Val-Asn-O-Gly) derived from AFP was compared to tamoxifen for activity against growth of human breast cancer xenografts implanted in immune-deficient mice. Both peptide and tamoxifen prevented growth of estrogen receptor-positive MCF-7 and T47D human breast cancer xenografts. A subline of MCF-7, made resistant to tamoxifen by a six-month exposure to this drug in culture, was found to be resistant to tamoxifen in vivo. Peptide completely prevented the xenograft growth of this tamoxifen-resistant subline of MCF-7. Neither peptide nor tamoxifen were effective in slowing the xenograft growth of the estrogen-receptor-negative MDA-M2-231 human breast cancer. In this study, tamoxifen was shown to stimulate the growth of the immature mouse uterus in vivo, and the peptide significantly inhibited tamoxifen's uterotrophic effect. The mechanism of action of peptide was different from that of tamoxifen in that the peptide does not interfere with the binding of (3H) estradiol to the estrogen receptor. In conclusion, AFP-derived peptide interfered with the growth of tamoxifen-sensitive as well as tamoxifen-resistant estrogen receptor-positive human breast cancers and thus it can be used in combination with or in place of tamoxifen for treatment of estrogen receptor-positive human breast cancers.

USE - (M1) is useful for diagnosing carcinoma, preferably primary and/or metastatic carcinoma, in a patient. (M1) is useful for diagnosing a cell-proliferative disorder such as breast cancer. (II) or (III) is useful for magnetic resonance imaging of a cell, tissue or patient which involves administering the agent to a cell, tissue or patient and rendering a magnetic resonance image of the cell, tissue or patient (claimed). (P1) and (P2) are useful for treating breast cancer, prostate cancer and other cancers which are affected by the steroid hormone/thyroid hormone super family of receptors.

ADMINISTRATION - In (M1), the administration of the labeled peptide is by parenteral injection chosen from intradermal, subcutaneous, intramuscular, intraperitoneal and intravenous injection (claimed).

EXAMPLE - Previous experiments had shown that an energy-minimized structure of octapeptide Glu-Met-Thr-Pro-Val-Asn-Pro-Gly (S1), indicated that the peptide had potential to form a horseshoe shaped structure. Energy-minimization studies of an analog of this peptide that would be generated by substitution of N-terminal Glu with Gln (Gln-Met-Thr-Pro-Val-Asn-Pro-Gly (S2)), indicated that this product had potential to bow even further inward and form a pseudo-cyclic structure. This pseudo-cyclic structure had greater structural stability due to hydrogen bonding between the N-terminal Gln gamma-carboxamide group and the C-terminal Gly alpha-carboxamide. This linear analog was therefore synthesized, and its biological activity was compared to (S1), in the estrogen-dependent immature mouse uterine growth assay. (S2) inhibited the estrogen-stimulated growth of mouse uterus with an optimal dose of 1 mug/mouse, similar to the native octapeptide (S1). These results suggested that the substitution of Glu to Gln did not detract from the biological activity and also did not change the biphasic nature of the dose-response curve. Shelf-life studies indicated that (S2) stored somewhat better than the native octapeptide, but its anti-estrotrophic activity also diminished to insignificant levels after five weeks of storage, indicating that the putative stabilization was not sufficient to prevent loss of biological activity during storage. Aged octapeptide (S2), stored in the **lyophilized** state at -20degreesC for over one year, was completely biologically inactive. However, brief treatment with 4 M urea restored its biological activity, suggesting that this peptide might have aggregated during storage, resulting in loss of biological activity. A scrambled form of the Q octapeptide had no biological activity either with or without urea treatment. The biological activity of stored inactive (S1) was likewise regenerated by 4 M urea. Gel filtration column chromatography of aged peptide (S2) yielded a single peak which became broader as a function of time in storage. This

suggested that small aggregate (dimers, trimers) were forming during storage. Although gel filtration chromatography has low resolution for monomers, dimers and trimers in this size range (841 Da to 2523 Da), the width of the peak suggested that aggregates might be separating from monomer. Fractions from different portions of the broad peak from aged, chromatographed peptide were therefore tested for biological activity. The higher molecular weight fraction was biologically inactive while the lower molecular weight fraction was active in the estrogen-dependent immature mouse uterine growth assay. This suggested that the octapeptide (S2), like its parent protein and precursor 34-mer peptide, aggregated during prolonged storage in the **lyophilized** state and only partially dissociated during chromatography, and that the monomeric form of the peptide was the active species. While not especially hydrophobic, the peptide does carry a net charge of only +1 at neutral pH, and taken together with the chromatography and urea evidence, it was reasonable to conclude that hydrophobicity played a role in its aggregation. In addition to aggregation, small peptides such as octapeptide (S1) or (S2), had structural flexibility that allowed them to attain a variety of different structural conformations. (85 pages)

L19 ANSWER 2 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-04160 BIOTECHDS
TITLE: New peptides derived from alpha-fetoprotein, useful for treating and diagnosing breast cancer, are significantly less toxic than tamoxifen or raloxifene;
peptide and antibody for use in disease therapy and diagnosis
AUTHOR: ANDERSEN T T; BENNETT J A; JACOBSON H I; MESFIN F B
PATENT ASSIGNEE: ANDERSEN T T; BENNETT J A; JACOBSON H I; MESFIN F B
PATENT INFO: US 2003170752 11 Sep 2003
APPLICATION INFO: US 2001-872623 2 Jun 2001
PRIORITY INFO: US 2001-872623 2 Jun 2001; US 2000-208614 1 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898258 [82]
AN 2004-04160 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A peptide (I) of 8-20 amino acids comprising a hydrophilic analog of an alpha-fetoprotein peptide having an 8 amino acid sequence, given in the specification, is new.

DETAILED DESCRIPTION - A peptide (I) of 8-20 amino acids comprising a hydrophilic analog of an alpha-fetoprotein peptide having Glu-Met-Thr-Pro-Val-Asn-Pro-Gly, is new. INDEPENDENT CLAIMS are also included for: (1) dimeric peptide consisting of two (I); (2) multimeric peptide consisting of three or more (I); (3) composition comprising (I) and a suitable carrier; and (4) antibody that specifically binds to (I).

BIOTECHNOLOGY - Preferred Peptide: The peptide is linear or cyclic. One or more of the amino acids is a (D)-amino acid. The peptide is labeled with a detectable marker, preferably a radiolabeled additional amino acid. Preferred Dimeric Peptide: The two peptides are Glu-Met-Thr-O-Val-Asn-O-Gly and Glu-Met-Thr-O-Val-Asn-O-Gly-Gln or Gln-Met-Thr-Pro-Val-Asn-Pro-Gly-Glu and Glu-Met-Thr-Pro-Val-Asn-O-Gly. O = hydroxyproline.

ACTIVITY - Cytostatic; Antirheumatic; Antiarthritic; Immunosuppressive; Antiinflammatory; Gynecological; Contraceptive. An **8-mer** peptide (EMTOVNOG sic) derived from alpha-fetoprotein (AFP) was compared to tamoxifen for activity against growth of human breast cancer xenografts implanted in immune-deficient mice. Both peptide and tamoxifen prevented growth of estrogen-receptor-positive MCF-7 and T47D human breast cancer xenografts. A subline of MCF-7, made resistant to tamoxifen by a six-month exposure to this drug in culture, was found to be resistant to tamoxifen in vivo. Peptide completely prevented the xenograft growth of this tamoxifen-resistant subline of MCF-7. Neither peptide nor tamoxifen were effective in slowing the xenograft growth of the estrogen-receptor-negative MDA-MB-231 human

breast cancer. A worrisome toxicity of tamoxifen is its hypertrophic effect on the uterus. In this study, tamoxifen was shown to stimulate the growth of the immature mouse uterus in vivo, and the peptide significantly inhibited tamoxifen's uterotrophic effect. The mechanism of action of peptide is different from that of tamoxifen in that the peptide does not interfere with the binding of (3H) estradiol to the estrogen receptor. In conclusion, AFP-derived peptide appears to be a novel agent that interferes with the growth of tamoxifen-sensitive as well as tamoxifen-resistant estrogen-receptor-positive human breast cancers, it inhibits the uterotrophic side effect of tamoxifen, and thus it can be used in combination with or in place of tamoxifen for treatment of estrogen-receptor-positive human breast cancers.

MECHANISM OF ACTION - Estrogen inhibitor.

USE - (I) is useful for reducing estrogen-stimulated growth in cells and treating and preventing cancer, preferably estrogen-dependent cancer, such as breast cancer. The peptides can be administered with tamoxifen (claimed). (I) is useful for treating prostate cancer and other cancers, which are affected by the steroid hormone/thyroid hormone superfamily of receptors. In addition (I) may be useful for treating inflammatory diseases such as rheumatoid arthritis, myasthenia gravis and organ transplant rejection. It is used against lymphoproliferative disorders would be appropriate. (I) may be useful in birth control, abortion, endometriosis, and menopause. (I) is also useful for detecting breast (or other) cancers.

EXAMPLE - Peptides were synthesized using FMOC solid phase peptide synthesis on a Pioneer Peptide Synthesis System (PerSeptive Biosystem, Inc., Framingham, Mass.) (see also Mesfin et al. 2000). Briefly, peptides were assembled on a solid support (Fmoc-Polyethylene-Graft Polystyrene Support) from the C-terminus, reacting the deblocked amino (N)-terminus of support-bound amino acid with the activated carboxyl (C)-terminus of the incoming amino acid to form an amide bond. Amino acids used in the synthesis had their N alpha-amino group protected by the 9-fluorenylmethoxycarbonyl (Fmoc) group, which was removed by piperidine at the end of each cycle in the synthesis. Side-chain protecting groups of amino acids were Asn(Trt), Gln(Trt), Glu(OtBu), Hyp(tBu), Thr(tBu) which were deprotected by trifluoroacetic acid (TFA) after peptide synthesis. The carboxyl-group of the amino acid was activated with HATU (O-(7-azabenzotriazol-1-yl)-1,1,3,- 3-tetramethyluronium hexafluorophosphate) obtained from PerSeptive Biosystems Inc. The specific amino acid derivatives, supports, and reagents used in the synthesis were purchased from PerSeptive Biosystems Inc. and NovaBiochem, San Diego, Calif. After synthesis was completed, the resin was washed 3 times with 100 % propanol and the cleavage reaction was achieved by incubating the resin in 10 ml trifluoracetic acid/thioanisole/anisole/1,2-ethanedithiol (90:5:2:3) per 0.5 g resin for 5 hours. The cleavage reaction mixture was filtered using a sintered glass funnel to separate the solid resin from the peptide solution. Filtrate volume was reduced to 1 ml by evaporation facilitated with a gentle stream of air and the peptides were precipitated by addition of 15 ml **dry**-ice chilled ethyl ether. The peptides were allowed to settle for five minutes at -80 degrees C, and the supernatant was aspirated. The peptides were then washed twice in similar manner with 15 ml of ethyl ether. After 3 further washings with 15 ml of ethyl acetate:diethylether (1.5:1, room temperature), the peptides were dissolved in deionized water, purified by reverse phase, **lyophilized**, and stored at -20 degrees C.
Cyclization of peptides was accomplished using methods described by Kates et al. (33 pages)

L19 ANSWER 3 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-08007 BIOTECHDS
TITLE: Novel oligonucleotide useful for interfering with the activity of a target nucleic acid molecule, and detecting pathogens, comprise a alkynyl functional group at C5 position of a pyrimidine heterocyclic base;
microarray detection device and liposome-mediated delivery

for gene therapy

AUTHOR: TURNER D H; BARNES T W
PATENT ASSIGNEE: UNIV ROCHESTER
PATENT INFO: WO 2002078619 10 Oct 2002
APPLICATION INFO: WO 2002-US4506 13 Feb 2002
PRIORITY INFO: US 2001-268429 13 Feb 2001; US 2001-268429 13 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-103235 [09]
AN 2003-08007 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An oligonucleotide (I) comprising first nucleotide (N1) comprising at least one alkynyl functional group (G1) at a C5 position of a pyrimidine heterocyclic base (II), and at least one second nucleotide (N2) covalently bound to N1 and comprising at least one G1 at C5 position of (II), is new.

DETAILED DESCRIPTION - An oligonucleotide (I) comprising a first nucleotide (N1) comprising at least one alkynyl functional group (G1) at a C5 position of a pyrimidine heterocyclic base (II), and at least one second nucleotide (N2) covalently bound to N1 and comprising at least one G1 at C5 position of (II), where (I) comprises: (a) loss in free energy of at least about 2 kcal/mol when: (i) G1 of N1 is removed from C5 position of (II), and (ii) (I) is covalently or non-covalently bound to nucleic acid molecule comprising nucleotide sequence that is substantially Watson-Crick complementary to sequence of (I); or (b) loss in free energy of at least about 2.8 kcal/mol when: (i) (I) is covalently or non-covalently bound to nucleic acid molecule comprising a nucleotide sequence that is less than substantially Watson-Crick complementary to (I); and (ii) N1 of (I) is covalently or non-covalently bound to a nucleotide of the nucleic acid which is not a Watson-Crick base pairing nucleotide for N1. INDEPENDENT CLAIMS are also included for the following: (1) A duplex (III) comprising a nucleic acid molecule and (I) hybridized to the nucleic acid molecule; (2) Designing (M1) an oligonucleotide capable of interfering with the function of a target nucleic acid molecule; (3) A microarray detection device (IV) comprising a substrate and several oligonucleotides bound to the substrate, each of the oligonucleotides comprising at least 6 nucleotide bases where 6 or more adjacent nucleotide bases of each are alkynylated; (4) Making (M2) a product; and (5) A self-assembling system (V) for preparing a product.

BIOTECHNOLOGY - Preferred Oligonucleotide: (I) does not bind to SV40 TAg mRNA. The oligonucleotide comprises a sequence of at least seven nucleotides comprising the first nucleotide, two second nucleotides, and at lest three other nucleotides comprising at least one alkynyl functional group at a C5 position of a pyrimidine heterocyclic base. Preferred Methods: M1 comprises identifying a target sequence of a target nucleic acid molecule and preparing an oligonucleotide comprising a nucleotide sequence that is substantially Watson-Crick complementary to the target sequence, the oligonucleotide including 6 or more adjacent nucleotide bases that are alkynylated in a manner which more favorably stabilizes the interaction of the oligonucleotide with the target nucleic acid molecule as compared to a second oligonucleotide that includes the same nucleotide sequence but lacks the 6 or more adjacent bases that are alkynylated. M1 further comprises determining the location of the 6 or more adjacent bases that will most favorably stabilize the interaction of the oligonucleotide with target nucleic acid. The determining step involves assessing the free energy potential of two or more sequences each comprising 6 or more adjacent alkynylated bases for hybridization to the target nucleic acid. M2 comprises introducing into a reaction medium a first nucleic acid molecule having bound to it a first molecule or compound and a second nucleic acid molecule having a second molecule or compound bound to it, the first and second nucleic acid molecules comprising substantially complementary nucleotide sequences that hybridize in the reaction medium and at least one of the first and second nucleic acid molecules comprising at least six adjacent alkynylated bases, where hybridization of the first and second nucleic acid molecules

brings the first molecule or compound into sufficient proximity to the second molecule or compound for the first and second molecules or compounds to form a product. In (M2), both the first and second nucleic acid molecules comprise at least two alkynylated bases. Preferred Device: In (IV), each of the several oligonucleotides comprises a same or different nucleotide sequence. The oligonucleotides comprise at least one set of oligonucleotides that hybridize to a first nucleic acid molecule. Preferably the oligonucleotides further comprise at least two sets of oligonucleotides that hybridize, respectively, to first and second target nucleic acid molecules. Preferred Self-assembling System: (V) comprises a first nucleic acid molecule comprising a first nucleotide sequence, the first nucleic acid molecule having a first molecule or compound bound on it, and a second nucleic acid molecule comprising a second nucleotide sequence which is substantially complementary to the first nucleotide sequence, the second nucleic acid molecule having a second molecule or compound bound on it, where at least one of the first and second nucleic acid molecules comprises at least two adjacent alkynylated bases, and where upon introduction of the first and second nucleic acid molecules into a reaction medium suitable for its hybridization, the first and second molecules or compounds are capable of self-assembly to form a product. In (V), both the first and second nucleic acid molecules comprise at least two alkynylated bases.

ACTIVITY - None given.

MECHANISM OF ACTION - Gene Therapy No supporting data is given.

USE - (I) is useful for interfering with the activity of a target nucleic acid molecule (an RNA molecule, DNA molecule or a natural or unnatural molecule of related structure, and is preferably not SV40 TAg mRNA) which comprises introducing into an in vitro or in vivo system including a target nucleic acid molecule, (I) which is effective to bind to the target nucleic acid molecule in a sufficient to interfere with its activity. (I) is useful for detecting the localization of a target nucleic acid molecule in an in vitro or in vivo system which comprises introducing into an in vitro or in vivo system, a labeled (I) including a nucleotide sequence which is substantially complementary and specific to a nucleotide sequence of a target nucleic acid molecule and having 6 or more adjacent nucleotide bases that are alkynylated, allowing sufficient time for the labeled (I) to hybridize with the target nucleic acid molecule, and determining the location of the labeled (I) in the system, the location of the labeled (I) being the same as the location of the target nucleic acid molecule. (IV) is useful for identifying an oligonucleotide having binding affinity for a target nucleic acid molecule comprising introducing a target nucleic acid molecule to (IV) under conditions effective for hybridization of substantially complementary sequences between the target nucleic acid molecule and the oligonucleotide, and detecting whether hybridization occurs between the target nucleic acid molecule and one or more several oligonucleotides bound to the substrate. The method further comprises identifying the nucleotide sequence and position of alkynylated bases in an oligonucleotide that hybridized to the target nucleic acid molecule. (IV) is also useful for detecting the presence of a target nucleic acid molecule (e.g., a target nucleic acid specific for a pathogen) in a sample which involves passing a sample over (IV) under conditions suitable for hybridization to occur between oligonucleotides and target nucleic acid molecules, and determining whether any target nucleic acid molecules hybridized to oligonucleotides during the passing (all claimed). (I) is useful for inhibiting activity of target nucleic acid where the inhibition is performed in vitro for research purposes for identifying viable targets, or in vivo providing a therapeutic or preventative treatment of a condition associated with activity of target nucleic acid molecule. By inhibiting activity of target nucleic acid molecule, (I) lessens the severity or altogether overcomes the condition or disorder associated with the activity of target nucleic acid molecule.

ADMINISTRATION - (I) is preferably administered by liposomes. No dosage given.

ADVANTAGE - (I) possess greater affinity and higher stability with

their target. The duplexes formed using (I) are stable at temperatures which would normally melt a duplex.

EXAMPLE - Synthesis of C5-(1-propynyl) pyrimidine oligodeoxynucleotides (PODN) was carried out as follows. Oligoribonucleotides were deblocked in ethanolic ammonia for 17 hours at 55degreesC. After filtering away support, RNA oligomers were incubated in 1 M triethylammonium hydrogen fluoride at 55degreesC for 50 hours. Solutions of crude products were evaporated, dissolved in water, and extracted against diethyl ether. After removing residual ether by evaporation, 5 mM aqueous ammonium acetate was added and the oligomers were desalted on a reverse phase Sep-Pak C-18 cartridge. The oligomers were purified by 20% polyacrylamide gel electrophoresis (PAGE). The product was UV visualized, cut out, and eluted with sterile water containing 0.5 mM Na2 ethylenediaminetetraacetic acid (EDTA). After separating the sample from a majority of the urea with a pre-packed PD-10 Sephadex column, the samples were dialyzed in an irradiated cellulose ester against 0.1 mM EDTA and subsequently against sterile water. Samples were then **lyophilized.** The purity of the oligonucleotides was greater than 95%. Product identity was confirmed for all RNA strands by electrospray mass spectroscopy. Thermodynamic parameters were measured for duplex formation between the DNA, d(5'CCUCCUU3'), or its fully propynylated analog and an RNA **7-mer**, r(3'GGAGGAA5'), that can form the same base pairs as the target sequence within the SV40 TAg mRNA. Full propynylation has a dramatic effect on duplex stability, DELTAGo37(DNA:RNA **7-mer**)=-7.6 kcal/mol whereas DELTAGo37(PODN:RNA **7-mer**)=-15.3 kcal/mol. Thus, full propynylation increases the equilibrium constant for duplex formation by more than 200,000-fold. The TM of the PODN:RNA **7-mer** duplex is 31.6 K higher than that of the unpropynylated duplex. To provide an empirical measure of stacking interactions possible for rA's in a duplex containing an all-purine RNA strand and an all-pyrimidine DNA strands, duplex stabilities were measured for d(5'CCUCCUU3'):r(3'AGGAGGAA5'), d(5'CCUCCUU3'):r(3'AGGAGGAAA5'), and their fully propynylated analogs. At 37degreesC, a 5' dangling rA on a DNA:RNA helix stabilized the duplex by 0.5 kcal/mol and a 3' dangling rA stabilized it by 1.3 kcal/mol. On a fully propynylated PODN:RNA duplex, however, they stabilized by 1.9 and 0.9 kcal/mol, respectively. Therefore, a 3' unpaired rA was more stabilizing than a 5' dangling rA in the unmodified duplex, while the reverse was true in the propynylated duplex. To provide an empirical measure of stacking interactions possible for dC's in a duplex containing an all-purine RNA strand and an all-pyrimidine DNA strand, duplex stabilities were measured for d(5'CCCUCCUU3'):r(3'GGAGGAA5'), d(5'CCUCCUUC3'):r(3'GGAGGAA5') and their fully propynylated analogs. An unmodified deoxycytidine stacking on the 5' or 3' end of the unmodified DNA:RNA helix stabilized duplex formation by 0.7 and 0.2 kcal/mol, respectively, while a dCp stacking on the 5' or 3' end of the fully modified PODN:RNA helix stabilized by 0.3 and 0.7 kcal/mol, respectively. Thus, effects of propynylation on stacking of dC are relatively small, and the dangling end providing more stabilization appears to switch from 5' to 3'. (86 pages)

L19 ANSWER 4 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09342 BIOTECHDS
TITLE: Polymer chip, useful e.g. for mutational analysis, comprises probes consisting of a fixed sequence and a degenerate wobble sequence that improves hybridization stability; DNA chip, DNA probe and DNA primer for mutant DNA detection
AUTHOR: ACHTMANN M; KIRCHNER R; MANN W; MORELLI G; ROPERS H
PATENT ASSIGNEE: MAX PLANCK GES FOERDERUNG WISSENSCHAFTEN
PATENT INFO: WO 2002004111 17 Jan 2002
APPLICATION INFO: WO 2000-EP7726 7 Jul 2000
PRIORITY INFO: DE 2000-1033091 7 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: German

OTHER SOURCE: WPI: 2002-171681 [22]
AN 2002-09342 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Polymer chip (A) comprising many probes (I), arranged at specific positions on a carrier and each with an oligomeric sequence consisting of a known number of monomers, is new. Each position supports many oligomeric sequences, each comprising a probe sequence (PS), the same for all (I) at a given position, and a wobble sequence (WS) comprising a permutation of monomers.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method for detecting nucleic acid using (A).

BIOTECHNOLOGY - Preferred Probes: At each position, all WS have the same number of monomers and all possible permutations of monomers are represented. Preferably, (I) also represents all possible permutations in PS. Particularly PS contains 4-10, best 6, nucleotides (nt) and WS at least 3, preferably 4-7, nt. (I) may be attached to the carrier through a spacer, specifically -NH-(T)10. Preferred Chip: Each position on the carrier also includes a binding component, specifically streptavidin, for immobilization of test material. Preferred Process: A nucleic acid test material is applied to (A) so that hybridization can occur, then polymerase chain reaction (PCR) performed so that probes are extended with incorporation of a labeled dNTP (deoxynucleoside triphosphate, where N is A, C, T, G, uridine or inosine). The label is particularly a fluorophore, e.g. Cy3, and detection is by fluorescent scanning. PCR conditions include hybridization at 30 degrees C, elongation at 50 degrees C and denaturation at 94 degrees C, and at least three cycles are performed, after which the mixture is cooled to e.g. 4 degrees C to inactivate polymerase. Efficient elongation occurs only where there is a perfect match between PS and the test sequence, but a least one mismatch can be tolerated in WS. The test nucleic acid includes a binding element, specifically biotin, for immobilization at the probe position, directly adjacent to (I). Particularly, biotin is introduced during amplification, from a biotinylated primer.

USE - (A) is used for detection of nucleic acid, particularly for mutational analysis in epidemiological studies.

ADVANTAGE - (A) is suitable for analysis of short sequences, especially incorporation of WS increases the number of monomers to a level sufficient for stable hybridization, but it does not affect specificity, which is still determined entirely by PS. (A) may include all possible permutations in PS, making it a universal chip. Mutational analysis can be performed simply, quickly and very efficiently, leading to rapid identification of even unknown sequence variations.

EXAMPLE - A polymer chip carried, at separate locations, probes containing 100 different hexameric sequences (HS) and having the structure 5'-(T)10-WS-HS, where WS represents all possible permutations of a **6-mer** sequence. Homologous gene segments from Neisseria meningitidis, of 300 base pairs, were amplified by polymerase chain reaction, then amplicons incubated with the chip, and three rounds of primer extension amplification performed, in presence of deoxyuridine triphosphate, labeled with the fluorophore Cy3. The chip was washed, **dried** and scanned, the most strongly fluorescent spots were complementary to TTCGCG (the sequence being tested for). (30 pages)

L19 ANSWER 5 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-16589 BIOTECHDS
TITLE: Identifying perfectly matched polynucleotide duplex, useful for various clinical applications including detecting genetic variations and pathogenic organisms, comprises cleaving duplex with mismatch-dependant endonuclease;
polynucleotide duplex identification, DNA probe and endonuclease for SNP detection and diagnosis
AUTHOR: DRMANAC S; XU C; QIAN X
PATENT ASSIGNEE: HYSEQ INC
PATENT INFO: US 2002048760 25 Apr 2002
APPLICATION INFO: US 1999-825408 10 Dec 1999

PRIORITY INFO: US 2001-825408 2 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-415021 [44]
AN 2002-16589 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying (M1) a perfectly matched polynucleotide duplex, comprising treating a duplex under conditions which result in a mis-match-dependent cleavage of at least one of the polynucleotides, and determining if cleavage has occurred, is new.

DETAILED DESCRIPTION - Identifying (M1) a perfectly matched polynucleotide duplex comprising: (a) providing a polynucleotide duplex comprising 2 polynucleotides; (b) treating the duplex under conditions which result in mismatch-dependent polynucleotide cleavage, where at least one of the polynucleotides will be cleaved in the duplex is mis-matched; and (c) determining whether at least one of the polynucleotides has been cleaved, where a lack of cleavage indicates that the duplex is perfectly matched, is new. INDEPENDENT CLAIMS are also included for the following: (1) releasing a detectable label bound by a polynucleotide to a solid substrate, where the label is only released if the polynucleotide is involved in a mismatched duplex with a second polynucleotide, comprising cleaving the polynucleotide in a mismatch-dependent manner so the label is released; and (2) determining the sequence of a target nucleic acid, comprising (a) contacting the target with a spatially-addressable array of immobilized probes of known sequence attached to a solid substrate and a detectably labeled oligonucleotide probe so a duplex forms between complementary polynucleotides; (b) covalently joining adjacently hybridized immobilized probes to form composite probes, thereby attaching the label to the solid substrate; (c) mismatch-dependent cleaving the probes to release the label from the solid substrate if the composite probe is in a mismatched duplex; and (d) identifying the labeled composite probes remaining attached to the solid substrate after cleavage.

BIOTECHNOLOGY - Preferred Method: In M1, the duplex comprises a polynucleotide attached to a solid substrate and a complementary polynucleotide which is detectably labeled, and polynucleotide cleavage is determined by determining whether the label is still associated with the solid substrate, with continued association indicating that the duplex is perfectly matched. Two polynucleotide may carry two labels, with cleavage determined by determining if the two labels have become decoupled. Cleavage is by a mismatch-specific endonuclease, preferably T4 endonuclease VII or CelI endonuclease, or a cocktail of endonucleases, or by a chemical cleavage reagent. The endonuclease(s) should be able to recognize a mismatch involving any of the naturally occurring nucleotide bases. The detectable label may be a fluorophore, radioisotope, enzyme, dye, electrophore mass labels (EML) or ligand, more preferably a fluorophore, most preferably 6-carboxytetramethylrhodamine (TAMRA) and is attached to a terminal nucleotide. When sequencing a nucleic acid, the sequence of the probes involved in uncleaved duplexes is determined by mass spectrometry. The probes form overlapping sequences so that the full sequence of a target nucleic acid can be determined.

USE - The method may be used to determine new sequences, as well as for the detection of mutations or single nucleotide polymorphisms. The methods have various clinical applications including detection of pathogenic organisms or deleterious genetic mutations.

EXAMPLE - A target nucleic acid, a 6-carboxytetramethylrhodamine (TAMRA)-labeled probe, and a series of **8-mer** oligonucleotides, including one perfectly matched to the target and others having single base mismatches were prepared suing standard techniques. Gene chips were prepared by fixing the **8-mer** probes to discrete locations on the surface of a glass slide to form an array of immobilized probes using standard techniques. The gene chips were hybridized to the target nucleic acid and labeling probe in the presence of T4 DNA ligase. Following hybridization/ligation, the chips were rinsed in cold buffer and spun **dry** to remove excess

target, labeling probe and ligase, then incubated with 125 H/micro liter T4 endonuclease VII in 75 mM PB buffer, 10 mM DTT (undefined), pH 6 for one hour, rinsed in 1% sarcosine, washed in 1% sarcosine and 4 x SSPE (undefined) wash buffer at 80 degrees C for one hour with shaking, rinsed with distilled water and spun **dry.** Control chips were not treated with the endonuclease. The chips were then scanned in a General Scanner with 90% PMT (undefined) and 90% laser power. The fluorescence of the TAMRA-label was detected by selecting Cy-3 channel in General Scanner 3000. In the absence of endonuclease all single base mismatches generated a strong signal, especially when the mismatch was located distant from the ligation site. Treatment with T4 endonuclease VII had little or no effect on the signal from the perfectly matched probe, but provided varying levels of mismatch discrimination for single base mismatches in all locations. (10 pages)

L19 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1966:447064 CAPLUS
DOCUMENT NUMBER: 65:47064
ORIGINAL REFERENCE NO.: 65:8725a-h,8726a-e
TITLE: (-)-(S)-2,2'-Diamino-4,4'-dimethoxy-6,6'-dimethylbiphenyl- configuration determination of the optically active 2,2'-diamino-biphenyls with rotatory dispersion and circular dichroism
AUTHOR(S): Musso, Hans; Steckelberg, Willi
CORPORATE SOURCE: Univ. Marburg, Germany
SOURCE: Justus Liebigs Annalen der Chemie (1966), 693, 187-96
CODEN: JLACBF; ISSN: 0075-4617
DOCUMENT TYPE: Journal
LANGUAGE: German

AB [2,6,4-RMe(MeO)C6H2]2(I) (R = NH2) (II) was separated with tartaric acid (III) into its antipodes. The absolute configuration of optically active II was determined by comparison of the optical rotatory dispersion and circular dichroism with (-)-(S)-[2,6-RMeC6H3]2 (IV) (R = NH2) (IVa) and their derivs. [(+)-(S)-I(R = NHAC) (V) with (+)-(S)-IV (R = NHAc) (VI) and (+)-(S)-I (R = N:CHC6H4OH-2) (VII) with (+)-(S)-IV (R = N:CHC6H4OH-2) (VIII). The results showed that both (+)-VII and (+)-VIII and also (-)-II and (-)- IVa have the same configuration. Since the configuration of (-)-IVa has been determined by chemical means as (S) (Mislow, CA 53,10134i), (-)-II must also have the (S) configuration. (All m.ps. are corrected). A solution of 200 mg. [2,4,**6-MeR**(MeO)C6H2]2 (IX) (R = NH2) (X) in 6 cc. H2O, 0.2 cc. concentrated HCl, and 0.3 cc. concentrated H2SO4 decolorized with C, diazotized with 103 mg. NaNO2 in 5 cc. H2O at 0°, added dropwise to a hot (100-10°) solution of 8 cc. concentrated H2SO4 and some CuSO4 in 20 cc. H2O, filtered while hot, coded, and let stand for a long time and the precipitate filtered and crystallized from H2O gave after drying in vacuo at 100° hydrated IX (R = OH) (XI), m. 164-5°, which sublimed in vacuo at 150° gave 60 mg. anhydrous XI, m. 200-2°. A solution of 2.123 g. (+)-(R)-Iva, [α]D 80.0° (c 1, Me2CO); .apprx.80% optical purity in 50 cc. 2N H2SO4 and 6 g. concentrated H2SO4 diazotized with 1.4 g. NaNO2 at 0°, added dropwise to 150 cc. hot (110°) 50% H2SO4, coded, and extracted with Et2O, the Et2O solution extracted with 2N NaOH, the alkaline solution acidified, the reddish precipitate (1.05 g.) filtered and dissolved in alkaline solution, the solution treated at 80° with Na2S2O4 (to remove azo dyes present), acidified, and extracted with Et2O, the extract evaporated, and the residue recrystd. twice from C6H6 gave 542 mg. (+)-(R)-IV (R = OH) (XII), m. 157-8°, [α]D 82.0° (c 1.0, Me2CO), 77.8° (c 0.6, EtOH). X (150 mg.) in 4 cc. H2O and 0.4 cc. concentrated HCl treated dropwise at 0° with 77 mg. NaNO2 in 0.5 cc. H2O, the solution poured into 10 cc. ice cold 30% H3PO2, refrigerated 18 hrs., and let stand 24 hrs. at room temperature, the precipitate filtered and dissolved in C6H6-petroleum ether, the solution washed with 10% aqueous NaOH and H2O and

evaporated, and the residue chromatographed on neutral silica gel with C6H6 gave 53 mg. (±)-XII, m. 120-2° (EtOH-dilute AcOH, sublimation in vacuo at 80°), which (107 mg.) methylated with 200 mg. Me2SO4 and 200 mg. K2CO3 in 20 cc. Me2CO gave 93 mg. (±)-IV (R = OMe) (XIII), m. 126° (EtOH-H2O). Similar methylation of 107 mg. (+)-(R)-XII gave 60 mg. (+)-(R)-XIII, m. 85-7°, [α]25D 52.7° (c 0.64, EtOH), at least 80% optical purity. Efforts to obtain the antipodes of (±)-X were unsuccessful, apparently because the salts of the antipodes differed too little in solubility and in crystal structure. To a stirred solution of 21.8 g. 3,5-O2N(MeO)-C6H3Me (XIIIa) in AcOH was added 3.3 g. NaOAc at room temperature (light excluded), followed dropwise 8 hrs. by 62 g. Br in 145 cc. AcOH while simultaneously adding portionwise 540 mg. Fe powder, after 1 hr. the solution poured into 11. ice H2O and let stand several hrs., and the precipitate filtered, washed with H2O, **dried**, and recrystd. from 200 cc. cyclohexane with C to give 24.7 g. 2,3,5-Br(O2N)(MeO)C6H2Me (XIV), m. 78-82°, sufficiently pure for further reaction; anal. XIV m. 80-2° (MeOH, sublimation in vacuo at 60°), N.M.R. (CDCl3) showing doublet at 7.05 (1H) and 7.4 ppm. (1H) [J = 3 cycles/sec. (c.p.s.)] (2 aromatic protons in meta position to each other); the mother liquors concentrated and the residue investigated by gas chromatography (4-m. steel column with SE-52; 30 cc. N/min., 230°; injection block 330°) showed the presence of XIIIa, XIV, 4-Br analog (XIVa) of XIV, 2,4,3,5-Br2(O2N)MeO)C6HMe (XV), and an unidentified compound (XVI) (retention times = 5.0, 9.5, 10.0, 13.5, and 19 min.); a very weak peak (<0.1%) at 11 min. could be assigned to the 6-Br analog of XIV. This residue chromatographed on silica gel with cyclohexane-C6H6 (initially 9:1, finally 1:1; 300 40-cc. fractions collected gave first XV followed by XIIIa with XIV and XIVa, while XVI appeared only after elution with C6H6. The compds. were all obtained anal. pure after recrystn. of the residues of the main fractions from cyclohexane (large losses); the proportion of the compds. in the mother liquor was 8:60:10:6:1 XIIIa-XIV-XIVa-XV-XVI. XIVa m. 105-6°. XV (3% yield) m. 155°. XVI, probably a dinitromethoxytoluene, m. 114-15°. In a larger experiment, chromatography of the mother liquors of XV gave 0.3% second dibromo compound, probably 2,6-Br2 analog of XV, m. 112-14°. A mixture of 10 g. XIV and 30 g. activated (with AcOH) Cu powder heated 2 hrs. at 200° and 3 hrs. at 230° (air excluded) and extracted with C6H6 and the extract chromatographed on silica gel with C6H6 gave from the first zone 4.8 g. I (R = NO2) (XVII), m. 115°. A mixture of 2.5 g. XIV and 3.5 g. Cu powder in 25 cc. PhNO2 heated 6 hrs. at 190° and PhNO2 steam distilled gave after chromatography (as above) 1 g. XVII, m. 113-15°. XVII (400 mg.) in 40 cc. warm MeOH hydrogenated over Raney Ni until the calculated amount H was absorbed gave 320 mg. (±)-II, m. 117-18°. A boiling solution of 8.2 g. (±)-II and 9.1 g. (-)-III in 40 cc. EtOH cooled slowly and let stand 24 hrs. and the precipitate repeatedly recrystd. from the smallest possible amount of 0.5M EtOH-(-)-III gave after 4 crystns. .apprx.2 g. salt, [α]25365 .apprx.-21° (c 4, EtOH), optical purity .apprx.25%, and after 6 crystns. .apprx.0.6 g. salt, [α]25365 .apprx.-37°, optical purity .apprx.45%. This fraction dissolved in 2N HCl and the solution treated with dilute aqueous NH3 with stirring and ice cooling gave 3-4% (-)-(S)-II, m. 134-6°, [α]25365 -167° (c 0.5, EtOH). Similar treatment of the free amine from all mother liquors with (+)III gave (+)-(R)-II, m. 134-6°, [α]25365 167° (c 0.5, EtOH). (±)-II (100 mg.) heated briefly with 2 cc. Ac2O, after several hrs. excess Ac2O decomposed with H2O, the solution evaporated in vacuo, and the residue **dried** (over KOH and chromatographed on silica gel with 4:1 C6H6-Et2O gave 122 mg. (±)-V, m. 63-8° (sublimation in vacuo at 120-30°). From (-)-(S)-II was similarly prepared 87% (+)-(S)-V, m. 70-5°, [α]25D 105° (c 0.7, EtOH). (±)-II (52 mg.) and 750 mg. 2-HOC6H4CHO (XVIII) heated 30 min. at 180-90°, the product taken up in EtOH, and the solution diluted with petroleum ether gave 65 mg. (±)-VII, m. 169-71° (C6H6cyclohexane). A solution of 60 mg.

(-)-(S)-II and 66 mg. XVIII in 3 cc. EtOH let stand 24 hrs. at room temperature deposited 89 mg. (+)-(S)-VII, m. 189-91°, [α]25D 500° (c 0.12, dioxane). (±)-II (170 mg.) in 4 cc. H2O and 0.4 cc. concentrated HCl treated at 0° with 86 mg. NANO2 in 0.5 cc. H2O, the solution poured into 10 cc. ice cold 30% H3PO2, kept 48 hrs. at 0° and 24 hrs. at room temperature, and extracted with EtOAc, the extract **dried** and evaporated, the residue chromatographed on silica gel with 1:1 C6H6-EtOAc, and the oily product from the first pale red zone distilled in vacuo at 80° gave 62 mg. (±)-IV (R = OMe) (XIX), m. 55°, identical (mixed m.p. and ir spectrum) with an authentic specimen. (±)-IVa (149 mg.) and 2.3 g. XVIII kept 20 min. at 185-90° and the mixture cooled and diluted with 8 cc. EtOH gave 240 mg. (±)-VIII, m. 234-6° (C6H6-EtOAc). Optically pure (-)-(S)-XIX (44 mg.) and 134 mg. XVIII in 5 cc. EtOH let stand 20 hrs. at room temperature gave 58 mg. (+)-(S)-VIII, m. 166°, [α]25D 627° (c 0.1, EtOH). The optical rotatory dispersion, circular dichroism, and uv spectra of (-)-(S)-IVa, (-)-(S)-II, (+)-(S)-V, (+)-(S)-VI, (+)-(S)-VII, and (+)-(S)-VIII were recorded.

L19 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1959:62632 CAPLUS
DOCUMENT NUMBER: 53:62632
ORIGINAL REFERENCE NO.: 53:11378d-i,11379a-b
TITLE: Polycyclic cinnoline derivatives. I. Reduction of 2,2'-dinitrobiaryls and related compounds
AUTHOR(S): Braithwaite, R. S. W.; Holt, P. F.; Hughes, A. N.
CORPORATE SOURCE: Univ. Reading, UK
SOURCE: Journal of the Chemical Society, Abstracts (1958) 4073-7
CODEN: JCSAAZ; ISSN: 0590-9791
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB To facilitate the preparation of polycinnoline derivs., the reduction of 2,2'-dinitrobiaryls (I), 5,6-dinitroacenaphthene (II), and 1,8-C10H6(NO2)2 (III) with LiAlH4, Zn in dilute alc. KOH, Na-Hg in MeOH or EtOH, dilute alc. Na2S, and Na polysulfide was investigated. Na-NO2 (20 g.) in 140 ml. concentrated H2SO4 at 0° added to 6,6'-dinitro-o-toluidine (IV), the mixture diluted with 120 ml. AcOH, poured into 10 g. Cu2Cl2 in 300 ml. concentrated HCl, 10 g. Cu-Br2 in 300 ml. 60% HBr, or 50 g. KI in 100 ml. H2O, the iodine removed with NaOH and Na2S2O3, the ppts. taken up in Me2CO, boiled with C, filtered, and the filtrate cooled and filtered gave the corresponding I, [3,4,**6-MeR**(O2N)C6H2]2 (R = Cl, Br, iodine, V, VI, VII), m. 217° (dilute Me2CO), 240° (dilute Me2CO), and 230-3° (C6H6), resp., in yields of 9.3, 9.9, and 9.3 g. I. (1.0 g.) in C6H6-Et2O was refluxed with excess LiAlH4 in Et2O, the excess hydride decomposed with H2O, the filtered solution concentrated, and the product crystallized (C6H6). The reduction converted IV into 0.15 g. 3,8-diamino-2,9-dimethylbenzo[c]cinnoline, 3,8-RRC14H10N2 (VIII) (R = NH2) (IX), m. 272-4° (decomposition), 6,6'-dinitrobenzidine (X) into 0.13 g. 3,8-diaminobenzo[c]cinnoline (XI), m. 265°, V into 0.6 g. VIII (R = Cl) (XII), m. 279°, VI into 0.69 g. VIII (R = Br) (XIII), m. 280°, and VII into 0.18 g. VIII (R = H) (XIV), m. 185°. Reduction of [2,6-(O2N)2C6H3]2 (XV) gave a polymeric azo compound, soluble in H2SO4 (d. 1.84) to a dark brown solution regenerating the polymer on dilution with H2O, soluble in strong aqueous NaOH to a purple solution regenerating the polymer on acidification. All samples gave ash. I. (1 g.) in hot alc. treated with 40% aqueous KOH, the solution boiled 10 min. with excess Zn dust, filtered, concentrated, diluted with water, the precipitate taken up in Me2CO or C6H6, boiled with C, the filtered solution concentrated, and cooled converted V into 0.6 g. XII, VI and VII into 0.16 g. XIV. I or XV (1 g.) in **dry** MeOH stirred several hrs. with excess Na-Hg, the mixture boiled, the filtered solution evaporated, and diluted with water reduced V to 0.23 g. XII, VI to 0.1 g.

XIII and 0.04 g. XIII 5-oxide (XVI), m. 284° (decomposition) (HCONMe2), VII to 0.23 g. XIV, XV to 0.26 g. [2,6-(H2N)2C6H3]2, m. 200° (Et2O or C6H6), also produced by reduction of [2,6-H2N(O2N)C6H3]2. The reduction of XV was carried out in EtOH with production of 0.4 g. black polymeric azo compound I (1 g.) in boiling 90% alc. refluxed 0.5-2.0 hrs. with aqueous Na2S, the mixture diluted with water, filtered, and the product recrystd. or purified by chromatography on Al2O3 transformed V into 0.3 g. XII 5-oxide, m. 275° (decomposition) (HCONMe2), VI into 0.27 g. XVI, VII into 31 mg. VIII (R = I) (XVII) 5-oxide, m. 242-6° (decomposition) (Me2CO), containing Me2CO and unstable to light. Reduction with NaOH added gave 37 mg. 2,9-di-methyl-3-iodobenzo[c]cinnoline 5-oxide, m. 212-15° (decomposition), but repetition of the reduction gave mixts. of mono- (mainly) and diiodo compds. III and XV gave impure polymeric azo compds. XV (1 g.) boiled with 1.5 g. Na2S and 1.5 g. S in hot alc., the product extracted with Et2O, the Et2O solution extracted with dilute HCl, and the acid solution basified and extracted with Et2O gave 0.14 g. 2-amino-2',6,6'-trinitrobiphenyl, m. 159-60° (alc.). Use of 10 g. Na2S and 3 g. S gave 87 mg. [2,6-H2N(O2N)C6H3]2 m. 241° (alc.). II (0.65 g.) in 300 ml. alc. and 20 ml. Me2CO refluxed with 0.9 g. NaOH in 10 ml. H2O and the solution acidified gave 0.5 g. black infusible polymeric azo compound Accordingly, direct reduction of I may lead to a polycyclic cinnoline derivative, its N-oxime, an amine, a nitroamine, or a polymer.

L19 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1958:65847 CAPLUS
DOCUMENT NUMBER: 52:65847
ORIGINAL REFERENCE NO.: 52:11846b-h
TITLE: Catalytic syntheses of pyridine bases in the vapor phase. X. Syntheses of 2-methyl-3-isoalkylpyridines
AUTHOR(S): Ishiguro, Takeo; Morita, Yoshio; Ikushima, Kazuo
CORPORATE SOURCE: Univ. Kyoto
SOURCE: Yakugaku Zasshi (1958), 78, 216-20
CODEN: YKKZAJ; ISSN: 0031-6903
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 51, 16463c. MeCOCH2R (I, R = alkyl group) (1 mole), 1.25-1.5 moles CH2:CHCH2OH, and 4-5 moles NH3 passed through Cd3(PO4)2-activated earth catalyst at 375-400° and the product fractionated yielded 2,3-MeRC5H3N (II) (R, % yield from I, b.p./mm., and m.p. of picrate given): MeCH2CH2 (III), 15, -, -; Me(CH2)3 (IV), 18, -, -; Me2CH (V), 5, 85-95°/14, 134-5°; MeCH2CHMe (VI), 5, 100-10°/15, 141-2°; Me2CHCH2 (VII), 16, 85-95°/16, 117-19°; Me2CHCH2CH2 (VIII), 16, 120-30°/14, 111.5-6.5°. Similarly, 0.45 mole CH2:CHCH2OH, 0.3 mole mesityl oxide, and 1.3 moles NH3 yielded 17.5 g. bases containing 6.8 g. 2-picoline, b16 70°, a small amount of 3-picoline, and 2.8 g. II (R = V), b16 75-85°. An equimolar mixture of AcCHRCO2Et and NH4NO3 at 0° saturated with **dry** NH3 gas, the solution in a sealed tube heated to 65-70° in 4 hrs., kept 3-8 hrs. at this temperature, cooled, the product extracted with Et2O, and distilled yielded MeC(NH2):CRCO2Et (IX) (R, % yield, b.p./mm., and m.p. given): III, 79, 120-5°/14, 43-4°; IV, 81, 134-5°/14, 38-40°; V, 39, 110-12°/14, 39.5-40.5°; VI, 32, 120-3°/16, -; VII, 75, 123-6°/14, 40-1°; VIII, 77, 140-2°/13, 53-4°. EtONa (40 ml. EtOH and 2.3 g. Na), 0.1 mole IX, and 0.1 mole CH2(CO2Et)2 in a sealed tube heated 4-10 hrs. at 140-50°, cooled, the solution filtered, the filtrate washed with Et2O, H2O added, the solution neutralized with dilute HCl, and the precipitate recrystd. from EtOH gave 2,3,4,6,5-MeR(HO)2(EtO2C)C5N (X) (R, % yield, and m.p. given): III, 35, 191-3°; IV, 38, 188-9°; V, 23, 220-1°; VI, 28, 186-6.5°; VII, 42, 190-2°; VIII, 34, 208°. X and 10% NaOH refluxed to give a clear solution, the solution acidified with dilute HCl, saturated AcOK solution added to neutrality (litmus),

heated 2 hrs. on an H2O bath, the precipitate filtered off, and recrystd. from dilute EtOH gave 2,3,4,**6-MeR**(HO)2C5HN (XI). XI (1 part) and 6 parts POCl3 refluxed 6 hrs., the POCl3 removed in vacuo, the residue made strongly alkaline with KOH, steam distilled, the distillate saturated with NaCl, the product extracted with Et2O, and distilled gave 2,3,4,6-MeRCl2C5HN (XII). Catalytic reduction of 2 parts XII and 1 part AcOK in 40 parts MeOH with 10% Pd-C yielded II. XII prepared were (R, % yield, and b.p./mm. given): III, 74, 130-1°/12; IV, 63, 140-1°/13; V, 55, 124-5°/14; VI, 49, 135-6.5°/15; VII, 65, 136-7°/15; VIII, 46, 143-5°/14. II prepared were (R and % yield from XII given): III, 83; IV, 82; V, 81; VI, 75; VII, 75; VIII, 75. XI prepared were (R, % yield from X, and m.p. of picrate given): III, 99, above 300°; IV, 96, above 300°; V, 97, 330° (decomposition); VI, 97, 330°; VII, 97, above 330°; VIII, 92, above 310°.

L19 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1958:50576 CAPLUS
DOCUMENT NUMBER: 52:50576
ORIGINAL REFERENCE NO.: 52:9093a-i,9094a-d
TITLE: 4',5-Dihydroxy-8-methylisoflavone, and a note on lotoflavin
AUTHOR(S): Whalley, W. B.
CORPORATE SOURCE: Univ. Liverpool, UK
SOURCE: Journal of the Chemical Society, Abstracts (1957) 1833-7
CODEN: JCSAAZ; ISSN: 0590-9791
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
GI For diagram(s), see printed CA Issue.
AB The identity of "tatoin", isolated from soya beans, with 4',5-dihydroxy-8-methylisoflavone (I) (R, R1, R2 = H, H, H) has been questioned (cf. Baker, et al., C.A. 49, 303i) and I has been synthesized. 2,4,5-(HO)2MeC6H2CO2H (25 g.) in 150 ml. MeOH containing 15 ml. H2SO4 refluxed 60 hrs., the solution evaporated in vacuo and the residue diluted with 100 ml. H2O, filtered and the crystalline residue recrystd. from dilute MeOH gave 15 g. 2,4,5-(HO)2MeC6H2CO2Me (II), m. 108°, also prepared in 90% yield by esterification of the acid with CH2N2. II (4.5 g.) in 40 ml. PhNO2 at 5°, 10 g. powdered AlCl3, and p-MeOC6H4CH2COCl (from 5 g. acid) in 25 ml. PhNO2 kept 12 days at 30°, worked up, the solid product repeatedly extracted with MeOH, and the exts. concentrated gave 2.1 g. crystalline 5-carbomethoxy-2,6-dihydroxy-4'-methoxy-3-methyldeoxybenzoin, 3,5,2,**6-MeR**(HO)R'O)C6HCOCH2C6H4OMe-4 (III) (R, R1 = CO2Me, H) (IIIa), m. 116°, intense red-brown ferric reaction in alc. IIIa (6.5 g.) in 50 ml. alc. and 50 ml. H2O containing 7 g. KOH refluxed 1.5 hrs. and crystallization of the product from MeOH gave 4.5 g. III (R, R1 = H,H) (IIIb), m. 164°, characteristic green ferric reaction, not identical with the isomeric 2,4-dihydroxy-4'-methoxy-5-methyldeoxybenzoin, 5,2,4-R(HO)2C6H2COCH2C6H4OMe-4 (where R = Me) (IV), m. 155° (mixed m.p., 120°). IIIb (2.8 g.) boiled 1 hr. with 1.5 g. Me2SO4 and K2CO3, the mixture extracted with Et2O, the extract washed with N NaOH, and the **dried** extract evaporated gave 2.1 g. oily III (R, R1 = H, Me) (IIIc), intense green ferric reaction in alc. IIIc (2.1 g.) kept 24 hrs. at 0° with 25 ml. HCO2Et and 2 g. Na dust, the oily product refluxed 5 min. in 15 ml. AcOH, the product chromatographed in C6H6 over Al2O3 and the purified product crystallized from dilute MeOH or C6H6-petr. ether (b. 60-80°) gave 1.1 g. I (R, R1, R2 = MeO, H,H) (Ia), m. 137°, no ferric reaction. Ia (0.4 g.) boiled 20 min. in 5 ml. HI (d. 1.7) and AcOH (from 2 ml. Ac2O) gave 0.3 g. I (R, R1, R2, = H,H,H) m. 180° (from dilute AcOH), intense green ferric reaction; diacetate, m. 175°. I (0.4 g.), 2 g. KOH, 2 ml. H2O, and 3 ml. MeOH heated and kept at 240° 40 min., the cooled mixture diluted with 25 ml. H2O and

acidified, the phenolic fraction isolated in the usual manner, and the product distilled at 160°/0.5 mm. gave 50 mg. authentic 2,4-HO(MeO)C6H3Me, thus unequivocally establishing the orientation of I. "Tatoin" and I and their derivs. have m.ps. (substance, diacetate, di-Me ether), 318, 185, 165° and 180, 174, 137°, resp. The non-identity is clearly established and it is probable, as stated by Baker, et al. (loc. cit.), that "tatoin" is identical with daidzein. Many abortive routes were explored. Interaction of p-MeOC6H4CH2MgBr with 2,6-(MeO)2C6H3CN gave unchanged nitrile together with (p-MeOC6H4CH2)2, m. 128° (MeOH), demethylated to (p-HOC6H4CH2)2, m. 198° (dilute MeOH). Refluxing 20 g. 4,6-dihydroisophthalic acid 100 hrs. in 300 ml. MeOH containing 25 ml. H2SO4, cooling and crystallizing the product from MeOH yielded 17 g. Me 4,6-dihydroxyisophthalate, m. 146°, which failed to react with p-MeOC6H4CH2COCl. Condensation of 5 g. 4-methylresorcinol (V) and 7 g. 4-MeOC6H4CH2CN in 125 ml. Et2O containing 4 g. ZnCl2 in 3 days, and crystallization of the product from petr. ether gave 4 g. 2,4-dihydroxy-4'-methoxy-5-methyldeoxybenzoin, 2,4,5-(RO)2R1C6H2COCH2C6H4Me-4 (R, R1 = H, Me) (VI), m. 154°, intense red-brown ferric reaction in alc. PhNO2 (80 ml.) containing 20 g. AlCl3 and 12 g. 2,4-(HO)2C6H3CO2Me at 0° treated 48 hrs. with 10 g. p-MeOC6H4CH2COCl in 25 ml. PhNO2, the product isolated and the PhNO2 recovered by steam-distillation, the semicryst. residue refluxed 30 min. in 400 ml. 4N KOH, and the product crystallized from dilute MeOH gave 8.5 g. VI (R,R1 = H, CO2H) (VIa), m. 200°, intense blood-red ferric reaction, methylated by boiling 4 hrs. in Me2CO with Me2SO4 and K2CO3 to give VI (R, R1 = MeO, CO2Me), m. 157° (MeOH), no ferric reaction. VIa (2 g.) boiled 5 min. in 15 ml. quinoline, containing 0.5 g. Cu bronze, and the product crystallized from C6H6 and dilute MeOH gave 0.45 g. VI (R, R1 = H,H), m. 158°. PhCH2Br (5.0 g.), 20 g. K2CO3, and 8 g. 3,2,4,6-Me(HO)2(MeO)C6HCOCH2C6H4OMe-4 (cf. Whalley, C.A. 48, 2699d) boiled 3 hrs. in 250 ml. Me2CO gave 8 g. 4-benzyl-2-hydroxy-4',6-dimethoxy-3-methyldeoxybenzoin, m. 118°, intense violet ferric reaction. The deoxybenzoin (7.5 g.) treated with 3 g. Na dust in 50 ml. HCO2Et cyclized to give 6.5 g. I (R, R1, R2 = MeO, PhCH2O, HO) was converted quantitatively into I (R, R1, R2 = MeO, PhCH2O, H) (Ic), m. 168° (from MeOH). Ic (3 g.) in 100 ml. AcOH catalytically debenzylated 1 hr. with Pd-C (0.4 PdCl4 and 1 g. C) in 5 ml. HCl gave 100% I (R, R1, R2 = MeO, HO, H); p-MeC6H4SO3H derivative (Id), m. 212° (Me2CO). Hydrogenolysis of Id for the elimination of the 7-HO group failed. Id (1 g.) in 2 l. MeOH containing 5 g. Raney Ni saturated 1 hr. with a rapid stream of H, and the concentrated solution fractionally crystallized gave 0.1-0.2 g. Id, 0.1 g. I (R, R1, R2 = MeO, HO, H), m. 260° (EtOH), and 0.1 g. I (R, R1, R2 = MeO, p-MeC6H4SO3, H), m. 149° (EtOH), no ferric reaction.

2,4-(HO)2C6H3CHO (40 g.) in 200 ml. H2O and 200 ml. concentrated HCl containing 200 g. Zn amalgam stirred vigorously 2 hrs. with addition of 50 ml. HCl, after 1 hr. the mixture kept overnight and decanted, the solution saturated with (NH4)2SO4 and exhaustively extracted with Et2O, and the extract distilled gave 19-20 g. V, m. 99-100°. C6H6 (100 ml.) containing 2.7 g. 2',4',5,7-tetramethoxyisoflavone (VII), m. 272°, intense red violet-brown ferric reaction, methylated by boiling 30 min. with Me2SO4-K2CO3 in Me2CO to almost quant. yield of 5-hydroxy-2',4',7-trimethoxyisoflavone, m. 154°, intense red-brown reaction; Ac derivative, m. 204° (MeOH), no ferric reaction. Synthesis of VII and its derivs. proved untenable the suggestion that the pigment lotoflavin, from Lotus arabicus, was VII (cf. Doporto, et al., C.A. 50, 10713e).

L19 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1956:57119 CAPLUS
DOCUMENT NUMBER: 50:57119
ORIGINAL REFERENCE NO.: 50:10781f-i

TITLE: New diarylmethanes
INVENTOR(S): Lambert, Arthur; Williams, Gwyn E.
PATENT ASSIGNEE(S): Imperial Chemical Industries Ltd.
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2732407		19560124	US	

AB 3,5,2-RMe(HO)C6H2CH2C6H2(OH)MeR'-2,5,3 (I) are prepared, where R is a tert-alkyl group of 4-8 C atoms and R' is a 1-alkylcycloalkyl group in which the cycloalkyl group is cyclopentyl, methylcyclopentyl, cyclohexyl, or methylcyclohexyl, where the 1-alkyl group has a maximum of 4 C atoms. I and its metal salts are useful as antioxidants for rubber, fats, and oils. I are prepared from equimol. amts. of 4,2,6-MeRR''C5H2OH and 4,2,**6**-**MeR**'R''C6H2OH, where R'' is H, CH2OH, or CH2Cl, and only one of the two R'' is not H. Thus, 1-methylcyclohexane and p-cresol combined in the presence of concentrated H2SO4 give 2-(1-methylcyclohexyl)-p-cresol (II), m. 60-1°. A stirred solution of II in 20 petr. ether (III) 20, 35% aqueous HCHO 20, and concentrated HCl 118 parts at 0-5° saturated with **dry** HCl, stirred another hr., the organic layer separated, washed with saturated brine, **dried** over CaCl2, 4,2-Me(Me3C)C6H3OH 30 in III 30 parts added, the mixture kept at room temperature 16 hrs., the solvent is distilled and the residue 30 min. heated at 140°, then dissolved in III 100 parts, washed, and distilled yields I(R = 1-methylcyclohexyl, R' = Me3C) (IV), crystals from III, m. 89-91°, b0.1 200-25°. Other examples of I, prepared like IV (R and R' given): Me3CCH2CMe2, 1-methylcyclohexyl, b1.0 235-45°; and Me3C, 1-methylcyclopentyl, b0.5 200-31°. Cf. C.A. 50, 5756e, 8735a.

L19 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1956:57120 CAPLUS
DOCUMENT NUMBER: 50:57120
ORIGINAL REFERENCE NO.: 50:10781f-i
TITLE: New diarylmethanes
INVENTOR(S): Lambert, Arthur; Williams, Gwyn E.
PATENT ASSIGNEE(S): Imperial Chemical Industries Ltd.
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
GB 749450		19560523	GB	

AB 3,5,2-RMe(HO)C6H2CH2C6H2(OH)MeR'-2,5,3 (I) are prepared, where R is a tert-alkyl group of 4-8 C atoms and R' is a 1-alkylcycloalkyl group in which the cycloalkyl group is cyclopentyl, methylcyclopentyl, cyclohexyl, or methylcyclohexyl, where the 1-alkyl group has a maximum of 4 C atoms. I and its metal salts are useful as antioxidants for rubber, fats, and oils. I are prepared from equimol. amts. of 4,2,6-MeRR''C5H2OH and 4,2,**6**-**MeR**'R''C6H2OH, where R'' is H, CH2OH, or CH2Cl, and only one of the two R'' is not H. Thus, 1-methylcyclohexane and p-cresol combined in the presence of concentrated H2SO4 give 2-(1-methylcyclohexyl)-p-cresol (II), m. 60-1°. A stirred solution of II in 20 petr. ether (III) 20, 35% aqueous HCHO 20, and concentrated HCl 118 parts at 0-5° saturated with **dry** HCl, stirred another hr., the organic layer separated, washed with saturated brine, **dried** over CaCl2, 4,2-Me(Me3C)C6H3OH 30 in III 30 parts added, the